

ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF
CHILD HEALTH AND HUMAN DEVELOPMENT
FISCAL YEAR 1974
VOLUME I

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

ANNUAL REPORT
OF
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NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT
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Volume I

ADMINISTRATIVE USE

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| | <u>Page</u> |
|---|-------------|
| Table of Contents | I |
| OFFICE OF THE DIRECTOR | |
| Summary Report | A-1 |
| <u>Office of Research Reporting</u> | |
| Summary Report | AA-1 |
| Activities at the Gerontology Research Center | AA-1 |
| Freedom of Information | AA-2 |
| Mongolism Education Campaign | AA-2 |
| Public Inquiries | AA-2 |
| Congressional and White House Activities | AA-3 |
| Science Writers' Seminars | AA-3 |
| <u>Office of Program Planning and Evaluation</u> | |
| Summary Report | AB-1 |
| OFFICE OF ASSOCIATE DIRECTOR FOR EPIDEMIOLOGY AND BIOMETRY | |
| Summary Report | B-1 |
| <u>Epidemiology Branch</u> | |
| Summary Report | BA-1 |
| Non-project Activities | BA-1 |
| Bibliography | BA-4 |
| Talks | BA-4 |
| Project Reports: | |
| HD-EB-3 Low-Weight-for-Gestational-Age in the U.S. | BA-6 |
| HD-EB-4 Relative Efficacy of Two Prenatal Care Regimens | BA-8 |
| HD-EB-10 Multiple Ovulation and Risk of Anencephaly and Spina Bifida | BA-10 |
| HD-EB-11 Infant Mortality Rates and Hardness of Local Water Supplies | BA-11 |
| HD-EB-12 Estimated Concordancy Rates for the Sudden Infant Death Syndrome Among Twins | BA-12 |
| HD-EB-14 Sudden Infant Death Syndrome in the United States: a Study of Geographic and Other Variables | BA-13 |
| HD-EB-15 Secular Trends in Twinning Rates and Infant Death Rates from Congenital Malformations | BA-14 |
| HD-EB-16 Potato Consumption and Neural Tube Malformations | BA-15 |
| HD-EB-17 Permutation Test for Spreads | BA-16 |
| HD-EB-18 Modified Rosenbaum's One and Two Layers Test | BA-17 |

Page

HD-EB-19 Monte Carlo Procedures for Modified Rosenbaum
Tests

BA-18

Contracts and Collaborative Research:

Relationship of Heterozygosity in Phenylketonuria to
Intelligence Quotient

BA-19

The Epidemiology of Birth Weight with Particular Reference
to the Effect of Induced Abortion on Subsequent Offspring

BA-20

Biometry Branch

Summary Report

BB-1

Specific Activities of the Branch

BB-1

Contracts

BB-4

Contracts and Collaborative Research:

Epidemiologic Study of Breast Cancer and Benign Breast
Lesions in Relation to the Use of Ovarian Hormones

BB-5

Birth Defects Monitoring Program

BB-6

Oral Contraceptives and Tumors of the Breast

BB-7

A Retrospective Study of the Risks for Cancer of the
Breast, Body of the Uterus, Ovary, and Cervix Among
Users of Contraceptives

BB-9

A Collaborative Study of Oral Contraception and Cerebro-
vascular Disease

BB-10

Publications

BB-11

Talks

BB-12

OFFICE OF ASSOCIATE DIRECTOR FOR PROGRAM SERVICES

Summary Report

C-1

Grants and Contracts Management Branch

Summary Report

CA-1

Negotiation of Research and Training Grants

CA-1

Research Grants

CA-1

Training Grants

CA-2

Fellowships

CA-2

New Research Training Fellowship Program

CA-2

Research Contracts

CA-2

Summary of Institute Supported Projects

CA-3

Program Statistics and Analysis Branch

Summary Report

CB-1

Program Analysis Section

CB-1

Statistical Analysis Section

CB-3

Information Systems Section

CB-5

NICHD Grants and Contracts Active During March 1974

CB-6

CENTER FOR POPULATION RESEARCH

Office of the Director

Summary Report

D-1

Behavioral Sciences Branch

Summary Report

DA-1

Socio-economic Correlates of Fertility

DA-3

Sex Roles and Socio-psychological Correlates of Fertility

DA-6

Demographic and Attitudinal Factors Affecting Family Formation

DA-9

Fertility, Abortion, Illegitimacy and Family Planning

DA-9

Migration, Social Mobility, and Residential Patterns

DA-12

Consequences of Population Growth

DA-14

Research Oriented Toward the Possible Development of

Population Policies

DA-15

Contract and Collaborative Research:

National Fertility Survey, 1970-1975

DA-16

A Study of Low Fertility Cohorts in the United States

DA-17

An Econometric Analysis of United States Fertility Change,
1940 to 1973

DA-18

Fertility Change After the Baby Boom: The Role of Economic
Stress, Female Employment, and Education

DA-19

The Impact of Economic, Demographic and Social Factors on
Fertility Trends in the United States, 1917-1972

DA-20

A Comparative Analysis of Economic, Age Structure and Sex Role
Determinants of Recent American Natality Trends

DA-21

An Investigation of the Determinants of Fertility in the
United States

DA-22

A Study of Fertility of Spanish-American Populations in the
United States and their Socio-economic Correlates

DA-23

Analysis of the Decline of Fertility in Europe, by Province
Human Fertility in Poland and the U.S.S.R.

DA-25

DA-26

Socio-economic Factors in the Reduction of Natality in the
Less Developed Areas

DA-28

Economic Cost and Value of Children in Four Societies

DA-29

Attitudinal Factors Affecting Family Size

DA-30

The Study of Fertility-related Decision-making Among
Married Couples

DA-32

Sex Role, Family Structure, and Fertility Control

DA-33

The Employed Woman: Family Planning and Careers

DA-35

Occupational Experience as a Determinant of Fertility

DA-36

A Study of Child-bearing and Labor Force Participation of
Women

DA-37

Women's Labor Force Participation and Fertility

DA-38

Urbanization, Migration and Fertility in Thailand

DA-40

Psychological Studies of Social Norms Influencing Family Size

DA-42

Explorations in Fertility Values

DA-43

Family, Career and Sexuality

DA-45

Longitudinal Analyses of Variables Predictive of Attitudes

Regarding Fertility and Actual Fertility Patterns

DA-46

| | <u>Page</u> |
|--|-------------|
| Age at Marriage and Time to First Birth: A Longitudinal Study of Parental Attitudes and Children's Personality | DA-47 |
| The Effect of Birth Order on Mother-child Relationship | DA-48 |
| A Review of Actual and Accepted Consequences of Family Size | DA-49 |
| A Study of Differentials and Trends in Marital Disruption, Remarriage, and the Fertility of Remarriage | DA-51 |
| Social-psychological Correlates of Urban Fertility | DA-53 |
| An Investigation of the Factors Involved in the Moral Judgment of Marital Status and Family Size | DA-55 |
| The Kin Network and Population Dynamics | DA-56 |
| The Effects of Purposive-Rationality, Traditionalism, and Economic Aspirations on the Process of Family Formation | DA-57 |
| The Work and Childcare Patterns of Women After the Birth of Their First Child | DA-58 |
| Relationship Between Age at Marriage, Birth Intervals and Total Fertility | DA-59 |
| Childspacing and Current Fertility: 1970 Census | DA-60 |
| Methods and Techniques for Using the 1960-1970 Census One Percent Public Use Samples | DA-61 |
| The Determinants of Fertility, Migration and Population Change of Counties in the United States, 1950 to 1960 | DA-62 |
| Study of Family Formation and Fertility - Key Trends and Patterns | DA-64 |
| New Estimation Techniques for Demographic Analysis | DA-65 |
| A Study of Physicians' Attitudes Toward Abortion | DA-66 |
| A Final Household Survey to Complete the Family Planning Evaluation Project | DA-67 |
| On the Consequences of Reproduction: A Utility Model of Reproductive Behavior | DA-68 |
| Development of Methodology for Evaluating the Demographic Impact of Organized Family Planning Programs on the United States | DA-69 |
| Determinants of Fertility Change in Tamil Nadu, India | DA-70 |
| A Study of Social Mobility and Fertility Control | DA-71 |
| Social and Psychological Factors Affecting Fertility, Family Planning and Clinic Utilization | DA-72 |
| An Analysis of the Causes and Patterns of Rural to Urban Migration of the Poor | DA-74 |
| Rates and Consequences of Population Change: The Growth and Decline of Counties in the Mountain Region | DA-76 |
| Review of Research Findings on Rural-Urban Migration with Annotated Bibliography | DA-77 |
| Analysis of Migratory Responses to Employment Opportunities | DA-78 |
| Income and Migration in the United States | DA-80 |
| An Analysis of the Spacing of Pregnancies, Births, and Completed Fertility in Relation to Socio-economic Status, Intergenerational Mobility and Aspirations for Children, Aberdeen, Scotland | DA-81 |
| The Distribution and Differentiation of Population Within Metropolitan Areas | DA-82 |
| Demography of the Black Population | DA-84 |

| | <u>Page</u> |
|---|-------------|
| A Study of Optimum Population Levels | DA-85 |
| The Economic and Environmental Consequences of Population Change | DA-86 |
| Population Growth and Employment Growth in Metropolitan Areas | DA-88 |
| Consequences of Metropolitan Population Stability or Decline | DA-89 |
| Population Redistribution and Depopulation in Non-metropolitan Pennsylvania: Social and Economic Correlates and Policy Implications | DA-90 |
| Support of Publication of <u>Population Index</u> | DA-91 |
| Processes of Demographic Development in Imperial Russia and the Soviet Union | DA-92 |
| Patterns of Emotions and Feelings in Sex-related behaviors | DA-94 |
| Correlates of Family Size and Childspacing in the United States and Puerto Rico | DA-95 |
| Role and Fertility Patterns of Urban Mothers | DA-96 |
| A Study of the Growth of Mexican-American Families | DA-97 |
| Fertility Dynamics of Cuban Refugees | DA-98 |
| Research on the Consequences of Childbearing and Childspacing Patterns | DA-99 |

Contraceptive Development Branch

Summary Report

| | |
|--|-------|
| A. Directed Fundamental (Biomedical) Research | DB-1 |
| Studies in the Female | DB-1 |
| Detection of Ovulation | DB-1 |
| The Ovum and the Oviduct | DB-2 |
| The Cervix | DB-3 |
| The Pituitary - Ovary - Corpus Luteum System | DB-3 |
| Prostaglandins | DB-4 |
| Studies of Male Reproduction Processes | DB-4 |
| Mechanism of Hormone Action | DB-6 |
| B. Product Development Research | DB-8 |
| New Drug Development Program | DB-8 |
| Testing Facility | DB-10 |
| Drug Delivery Systems | DB-11 |
| Development of Sterilization Techniques (Female) | DB-12 |
| Development of Sterilization Techniques (Male) | DB-12 |
| Clinical Studies (Female) | DB-13 |
| Clinical Studies (Male) | DB-13 |
| Hormone Distribution | DB-14 |
| References | DB-15 |

Contract and Collaborative Research:

| | |
|---|-------|
| Detection of Ovulation in Chronically Instrumented Animals | DB-20 |
| Development of Techniques for the Detection of Ovulation in the Primates | DB-22 |
| Development of Techniques for Study of Oviduct Transport and Ovulation in Primates | DB-23 |
| An Interdisciplinary Program Project on the Biology of the Oviduct and Gamete Transport | DB-24 |
| A Bioengineering Approach to the Study of Tubal Activity and Contraception | DB-26 |

| | <u>Page</u> |
|---|-------------|
| Regulation of Bicarbonate in Oviductal Fluid | DB-27 |
| Physiology of the Oviduct | DB-28 |
| Oviductal Fluid in the Rat and Mouse: A Study of the Chemical and Physical Properties and Mechanism of Formation | DB-29 |
| Morphochemical Analysis of Oviductal Mucins | DB-30 |
| Genetic Expression in Mammalian Embryo | DB-32 |
| Immunopharmacology of the Human Fallopian Tube | DB-33 |
| Oviduct Cilia: Structure, Function, Development | DB-34 |
| Ultrastructural Studies on the Oviduct of the Intact Normal, the Ovariectomized and Ovariectomized-Hormone Treated Rabbit and Pig-tailed Monkey, <i>Macaca-nemestrina</i> | DB-35 |
| Anti-luteinizing Activity of Follicular Ovum | DB-37 |
| Endocrine Control of Human Oocyte Maturation | DB-38 |
| Fertilization of Mouse Ova <u>in vitro</u> by Epididymal Sperm | DB-39 |
| Morpho-physiologic Studies on Reproductive Organs and Germinal Cells of Free Ranging Male and Female Macaques | DB-41 |
| The Effects of Intrauterine and Embryonic Elevation on Pregnancy in Experimental Animals | DB-42 |
| Progesterone and Uterine Secretion | DB-43 |
| Steroid Hormone Receptors in Human Cervix | DB-44 |
| Sex Hormone Receptors in the Cervix and Cervical Response to Sex Hormones | DB-45 |
| Estrogen and Progesterone Receptors in the Cervix During the Menstrual Cycle of the Chimpanzee | DB-46 |
| Coordinated Approach to the Physicochemical Characteriza- tion of Human Cervical Mucus | DB-47 |
| Biochemical Characteristics of Cervical Mucus of <i>Macaca radiata</i> (Bonnet Monkey) during Menstrual Cycle | DB-48 |
| Development of an <u>in vitro</u> Model System for the Study of the Dynamics of Sperm Transport Through the Human Cervical Canal | DB-49 |
| Studies on Sperm Transport Through the Cervix | DB-50 |
| Sperm Antibody Activity in Cervical Mucus of Women | DB-51 |
| Development of a Laboratory Screening Procedure for Inhibitors of Progesterone Synthesis | DB-52 |
| The Study of Neural Regulation of Ovarian Function | DB-54 |
| Pilot Study for the Large Scale Preparation of Purified Human Chorionic Gonadotropin | DB-55 |
| New Methods of Fertility Regulation: Specific Binding of Luteinizing Hormone to Cells of the Corpus Luteum and Inhibition of this Process | DB-56 |
| Development of Radioimmunoassays for Rhesus Monkey Gonadotropins | DB-57 |
| Development of an Automated Immunoradiometric Assay System | DB-58 |
| Control of Ovulation in the Monkey | DB-59 |
| Endocrine Regulation of the Corpus Luteum | DB-60 |
| The Control of Ovulation and Corpus Luteum Function | DB-61 |
| Regulation of the Changes in Corpus Luteum Physiology | DB-62 |
| A Simultaneous Theoretical and Empirical Approach to the Study of the Rat Estrous Cycle | DB-63 |
| Effect of Prostaglandins on Ovarian Function | DB-64 |
| Isolation of a Bovine Uterine Luteolytic Factor | DB-65 |

| | <u>Page</u> |
|--|-------------|
| Relationships of Endometrial Prostaglandins and Luteal Function | DB-66 |
| Molecular Biology of the Human Corpus Luteum | DB-67 |
| New Methods for Fertility Regulation | DB-68 |
| Prostaglandin: Its Role and Effect in Contraception | DB-70 |
| Quantification of Prostaglandins | DB-72 |
| The Role of Prostaglandins in the Secretion of Gonadotropins | DB-74 |
| The Role of Prostaglandins in Ovarian Physiology | DB-76 |
| Enzymes of the Sperm Acrosomes, Occurrence of the Inhibitors of These Enzymes in Seminal Plasma and the Role of the Enzymes and Inhibitors in Capacitation of Sperm and in the Penetration and Fertilization of Ova | DB-77 |
| Biochemical Requirements for Fertilization of Mammalian Ova | DB-79 |
| Study on Human Acrosomal Proteinase | DB-81 |
| Control of Sperm Fertilizing Capacity | DB-82 |
| Role of the Epididymis in Sperm Maturation | DB-84 |
| Hormonal Control of Sperm Maturation | DB-85 |
| Hormonal Control of the Epididymis | DB-87 |
| Androgen Receptors in the Testis | DB-88 |
| Morphological and Experimental Studies on Spermatogenesis, Sperm Maturation, and Means of Inducing Reversible Infertility in the Male | DB-89 |
| Metabolism of Sperm Nuclear Proteins During Spermiogenesis | DB-91 |
| Ultrastructural and Electron Cytochemical Studies of Mammalian Spermiogenesis | DB-93 |
| Ultrastructural Changes in the Sertoli Cells in Rats Under Various Hormonal Conditions | DB-94 |
| Dynamics of Spermatogenesis: Quantitative Morphological and Physiological Characterization of Isolated Meiotic Cells | DB-96 |
| Ultrastructural and Immunological Investigation of Spermato- genesis, Sperm Transport, and Fertilization in the Mammal | DB-98 |
| Androgen Polydimethyl-siloxane Implants: Metabolic Fate of Testosterone and Contraceptive Efficacy of Different Androgens | DB-99 |
| Fine Structure and Function of the Accessory Glands of the Male Reproductive Tract | DB-100 |
| Study of Mammalian Spermatozoa | DB-102 |
| Immunologic Localization of Prostaglandins in the Reproductive Tract of Rabbit | DB-103 |
| Formation of Dihydrotestosterone in Male Sex Organs and Transport of Steroid Androgens from the Testis to the Epididymis | DB-104 |
| Assay of Progesterone Receptor Activity in the Guinea Pig Uterus | DB-105 |
| Studies on Steroid Hormone Receptors and their Role in Reproductive Biology | DB-106 |
| The Study of Progesterone-binding Proteins as a Tool for the Detection of Progesterone Antagonists and Agonists | DB-107 |
| Estrogen-Receptor Substances of Oviduct Tissue | DB-109 |
| A Model for Studying Inhibition of LH Action on the Corpus Luteum | DB-110 |
| Biochemistry and Mechanism of Action of Human Gonadotropins | DB-111 |

| | <u>Page</u> |
|---|-------------|
| Steroid Hormone Binding in Reproductive Organs | DB-112 |
| Impeding Estrogens as Contraceptives - Mechanism of Action | DB-113 |
| Hormonal Requirement for Implantation in the Rhesus Monkey (Macaca mulatta) | DB-114 |
| Synthesis and Testing of Antifertility Compounds | DB-116 |
| Synthesis of Prostaglandin Analogs for Biological Studies | DB-117 |
| Production of New Type of Contraceptives Based on Derivatives of Luteinizing Hormone-releasing Hormone (LH-RH) | DB-118 |
| Contract Program for Obtaining, Synthesizing, and Testing Antifertility Compounds | DB-119 |
| Blastocidal and Spermicidal Action of Extracts and Compounds from Endod (Phytolacea Dodecandra) | DB-120 |
| The Development of Inhibitors to Estrogen Biosynthesis | DB-121 |
| Synthesize and Furnish Steroidal Compounds with Anti- fertility Activity | DB-123 |
| The Synthesis and Testing of Silicon Substituted Steroids as Potential Antifertility Compounds | DB-124 |
| The Synthesis of Potential New Agents for the Control of Fertility | DB-125 |
| Synthesize and Furnish Peptide Inhibitors of Luteinizing Hormone-releasing Factor/Follicle Stimulating Hormone- releasing Factor | DB-126 |
| Synthesize Novel Derivatives of Progesterone and Testosterone | DB-127 |
| Synthesis of Prostaglandin Analogs | DB-128 |
| Structure-activity Studies of Small Peptides which Release LH and FSH | DB-129 |
| Synthesize Nitrogen-containing Analogs of Prostaglandins | DB-131 |
| Biological Testing of Thyrotropin Releasing Hormone (TRH) to Determine the Extent and Nature of Antifertility and other Endocrine Activity | DB-132 |
| Synthesis of Novel Steroids for Fertility and Population Control | DB-133 |
| Synthesis and Testing of Potential Inhibitors of Luteinizing Hormone Releasing Factor | DB-134 |
| Synthesis of 12 α -Methyl Prostaglandins Related to PGF ₂ α | DB-135 |
| Synthesis of Novel Compounds of Potential Antifertility Activity | DB-136 |
| Novel Steroids as Potential Antifertility Agents | DB-137 |
| Synthesis of Ring-A Bi-and Tricyclo Steroids with Potential Antifertility Activity | DB-138 |
| Study of Compounds for Inhibition of Spermatogenesis | DB-139 |
| Synthesis of Steroidal Compounds Possessing a Bridged Bicyclic A-ring with Potential Antifertility Activity | DB-141 |
| Synthesis and Testing of Irreversible Inhibitors of Uterine Progesterone Binding Factors. Synthesis and Testing of Irreversible Inhibitors of Uterine Estradiol Binding Factors | DB-142 |
| Novel Steroidal Gestagens | DB-143 |
| The Synthesis of Novel Steroids with Potential Antifertility Activity | DB-144 |
| Non-steroidal Nitroheterocycles with Potential Antifertility Activity | DB-145 |

| | <u>Page</u> |
|--|-------------|
| Synthesis and Testing of Antifertility Compounds | DB-146 |
| Synthesis of Antifertility Compounds New and Novel | DB-147 |
| Synthesis and Testing of Antifertility Compounds | DB-148 |
| Synthesis of Luteolytic Prostanoids | DB-149 |
| The Synthesis and Testing of Antiprogestational Agents Derived from 7(8 \rightarrow 11 α) <u>Abeo</u> -steroids | DB-150 |
| The Synthesis and Testing of Prostaglandin Synthetase Inhibitors with Potential Antifertility Activity | DB-151 |
| The Synthesis and Testing of Some Novel 11-Oxa-16- Substituted Corticosteroids as Potential Antifertility Agents | DB-152 |
| Synthesis of Seco and Nor Analogs of Norethindrone and Progesterone | DB-153 |
| The Synthesis of 15-Phosphasteroids for Antifertility Screening | DB-154 |
| Synthesis of Ring-C Aromatic Steroids as Potential Antifertility Agents | DB-155 |
| Biological Testing Facility for the Evaluation of New Antifertility Drugs and Devices | DB-156 |
| Implantable Bio-absorbable Capsules in Slow-release Contraceptive Drug Delivery Systems | DB-157 |
| Development and Evaluation of a Biodegradable Drug Delivery System | DB-158 |
| A Study of Biodegradable Polymers for the Sustained Delivery of Contraceptive Drugs | DB-159 |
| Development and Testing of an Implantable Contraceptive Delivery System | DB-160 |
| Intrauterine Release of Estriol for Contraception | DB-162 |
| Polyelectrolyte Delivery Systems for Chemical Cauterization and Sustained Release | DB-163 |
| The Study of Intrauterine Microcapsules as a Prolonged Drug Release Form | DB-164 |
| A Study of the Factors that Control Diffusion Rates in Controlled-release Contraceptive Systems | DB-166 |
| Systems Approach to Vaginal Delivery of Contraceptive Drugs - Methodology and Mechanism for Absorption | DB-168 |
| Development of an Intra-uterine Sterilization System for the Female | DB-169 |
| Device and Technique for Blocking Fallopian Tubes | DB-171 |
| Development of a Reversible Vas-occluding Prosthesis Utilizing a Microporous Tissue Interface | DB-173 |
| Design and Development of Implantable Contraceptive Devices for Use in the Male | DB-174 |
| Development of New Sterilization Techniques | DB-176 |
| The Use of Reversible Vasectomy Device in the Guinea Pig | DB-177 |
| Clinical Studies of Estrogens as Postcoital Contraceptive Agents | DB-178 |
| Clinical Studies of Estrogens as Postcoital Contraceptive Agents | DB-179 |
| Clinical Investigation of Ethynyl Estradiol and Conjugated Natural Estrogens as Postcoital Contraceptive Agents | DB-180 |

| | <u>Page</u> |
|--|-------------|
| Study of the Safety and Efficacy of a Conjugated Estrogen Preparation as a Postcoital Contraceptive | DB-181 |
| Clinical Evaluation of a Fluid-filled Intrauterine Device | DB-182 |
| Contraceptive Development Studies for Males: Oral Steroid Hormone Administration | DB-183 |
| Distribution of Hormonal Substances | DB-184 |
| | |
| <u>Population and Reproduction Grants Branch</u> | |
| Summary Report | DC-1 |
| Population Research Center Award Program | DC-1 |
| Program Project Grants | DC-2 |
| Research Career Program (RCP) | DC-3 |
| Fellowship Programs | DC-3 |
| Training Programs | DC-3 |
| Staff | DC-4 |
| Symposia and Workshops | DC-4 |
| Highlights of PRGB Supported Biomedical Research | DC-5 |
| Highlights of PRGB Supported Behavioral-Social Research | DC-9 |
| Fertility | DC-10 |
| Population Growth, Movement, and Composition | DC-11 |
| Family and Women's Status | DC-11 |
| Population Policy | DC-12 |
| | |
| <u>Fertility Regulating Methods Evaluation Branch</u> | |
| Summary Report | DD-1 |
| | |
| Contract and Collaborative Research: | |
| Contraceptive Drug Study | DD-9 |
| Effects of Steroid Contraception on Cervical Dysplasia | DD-10 |
| A Five-year Prospective Study of Abnormal Cervical Cytology | DD-11 |
| An Epidemiologic Case-control Study of Thromboembolism Related to the Use of Oral Contraceptives | DD-12 |
| A Collaborative Study of Oral Contraception and Cerebrovascular Disease | DD-14 |
| Effects of Contraceptives on Blood Coagulation | DD-16 |
| Effect of Oral Contraceptives and Conjugated Estrogens on Renin Activity and Aldosterone | DD-19 |
| Endogenous and Exogenous Sex Steroids and Nutritional Status | DD-22 |
| Interaction of Contraceptive Steroids with Essential Dietary Nutrients | DD-24 |
| Relationships of Contraceptive Steroids to Nutritive State | DD-25 |
| Evaluation of Effects of Oral Contraceptives on Vitamin B ₆ Nutrition | DD-27 |
| Interactions of Contraceptive Steroids with Metabolic Functions of Vitamin B ₆ | DD-29 |
| Relationship of Effect of Oral Contraceptives on Blood Lipids and Nutritional Status | DD-31 |
| The Effects of Contraceptive Steroids on Trace Elements | DD-32 |
| Investigation of a Possible Interaction of Contraceptive Steroids with Vitamin E and Polyunsaturated Fatty Acids | DD-34 |

| | <u>Page</u> |
|---|-------------|
| Biochemical Mechanisms of Contraceptive Steroid-induced Abnormalities in Absorption, Metabolism and Function of Vitamin K | DD-35 |
| Effect of Oral Contraceptive Steroids on Calcium, Phosphorus, Magnesium and Zinc Metabolism, with Emphasis on Bone Mineralization | DD-37 |
| The Interaction of B-Vitamins with Contraceptive Steroids in Female Rats | DD-39 |
| The Effects of Oral Contraceptive Steroid Hormones on Folic Acid Metabolism | DD-41 |
| Prospective Study of the Effects of Contraceptive Agents, Related Methods, and Procedures on the Frequency of Birth Defects | DD-43 |
| Contraceptive Agents and Birth Defects | DD-44 |
| Methods of Contraception, Pregnancy Tests, and Congenital Malformations: An Epidemiological Study | DD-47 |
| The Effect of Oral and Other Contraceptives on Congenital Abnormalities and Outcome of Pregnancy | DD-48 |
| Effects of Contraceptive Agents, Related Methods and Procedures and Frequency of Birth Defects | DD-50 |
| Evaluation of the Effects of Contraceptive Agents, Related Methods and Procedures and Frequency of Birth Defects | DD-51 |
| Level of Risk of Female and Male Offspring of Mothers Exposed to Ingestion of Diethylstilbestrol During Pregnancy | DD-53 |
| Consequences of Contraceptive Failure in Terms of Birth Weight, Fetal Loss, Congenital Malformations, and Infant Mortality | DD-55 |
| Effects of Vasectomy on Testes, Epididymis and Vas Deferens | DD-57 |
| An Immunogenetic Analysis of Sperm Autoantigens in the Mouse | DD-58 |
| Immunologic and Morphologic Consequences of Vasectomy | DD-59 |
| Vasectomy and Autoimmune Disease | DD-61 |
| Immunologic Studies on Steroid-producing Cells | DD-62 |
| Endocrine Changes in Vasectomized Men | DD-63 |
| Ultrastructural and Immunochemical Studies of Sperm Antigens Involved in the Autoimmune Response in Vasectomized Mammals | DD-64 |
| Sperm Immunology | DD-65 |
| Autoimmune Phenomena and Mechanisms in Human Vasectomy | DD-67 |
| Immunopathologic Consequences of Vasectomy | DD-69 |
| USC Collaborative Study of the Effect of Vasectomy | DD-71 |
| Immunologic and Morphologic Effect of Vasectomy in Monkeys | DD-72 |
| Immune Alterations Associated with Vasectomy | DD-73 |
| Peripheral Metabolic Effects of the Ethynyl Estrogens in Subhuman Primates and Canines as Compared to Man | DD-74 |
| Biliary and Urinary Excretion, Enterohepatic Circulation, and Hepatic Effects of Contraceptive Steroids | DD-75 |
| Contraceptive Steroids in Beagle Dogs | DD-77 |
| Metabolism of Contraceptive Synthetic Estrogens | DD-78 |
| Central Repository for Tissue Evaluation of Contraceptive Steroids | DD-80 |
| Comparative Pharmacology of Steroid Contraceptive Drugs | DD-81 |

| | <u>Page</u> |
|---|-------------|
| The Baboon: An Animal Model for the Study of Contraceptive Steroids | DD-83 |
| Pharmacopathologic Effect of a 17 Δ -Hydroxyprogesterone Derivative in Beagle Bitches | DD-84 |
| Effects of Commonly Used Drugs on the Metabolism of Ethinyl Estradiol and Mestranol | DD-85 |
| A Study of the Metabolism of Steroidal Oral Contraceptives | DD-86 |
| Metabolism and Metabolic Effects of Steroidal Oral Contraceptives | DD-87 |
| Postcontraceptive Reproduction in Man | DD-88 |
| A Comparative Study of Progestin Metabolism in Animals | DD-89 |
| Development of Radioimmunoassays for Norethindrone, Norgestrel, Ethinyl Estradiol and Mestranol | DD-91 |
| The Metabolism of Derivatives of 17 Δ -Acetoxypogesterone in Beagles and Rhesus Monkeys | DD-92 |

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAMS

Summary Report

E-1

Growth and Development Branch

Summary Report

EA-1

Mechanisms of Biological Growth

EA-2

Immunologic Mechanisms and Pharmacological Studies in Development

EA-3

Nutrition

EA-5

Physical Growth

EA-7

Developmental Behavioral Biology

EA-8

Learning and Cognitive Development

EA-9

Human Communication Research

EA-9

Personality and Social Development

EA-10

Adolescence

EA-11

Research Training Activities

EA-13

Contract and Collaborative Research:

Study of Relationship Between Nutrition and Behavioral Development

EA-16

A Study of Malnutrition and Mental Development in Bogota, Colombia

EA-17

Research Materials and Assistance for Studies of Language Development in Children

EA-18

Prediction of Mature Stature in Growing Children

EA-19

Immunologic Responses of Normal and Malnourished Infants to Standard Antigens

EA-20

Analysis of Data from an Ecological Study of Infection, Malnutrition, and Growth of Children in a Guatemalan Indian Village

EA-21

Non-human Primate Colony

EA-22

Instrumentation Design for Electrophysiological Studies

EA-23

Interactions Between Malnutrition and Infection: Anti-bacterial Defenses in the Newborn and Responses to

Measles Virus in Malnourished Children

EA-24

| | <u>Page</u> |
|---|-------------|
| <u>Perinatal Biology and Infant Mortality Branch</u> | |
| Summary Report | EB-1 |
| Pregnancy and Maternal Health | EB-2 |
| NICHD Grants and Contracts Active During April 1974, PBIM | EB-3 |
| Developmental Biology | EB-5 |
| Fetal Health and Development | EB-6 |
| Infant Survival and Well Being | EB-8 |
| Sudden Infant Death Syndrome | EB-9 |
| Conferences | EB-15 |
| Publications | EB-16 |
| Foreign Country Activities | EB-17 |
| INSERM | EB-17 |
| Personnel | EB-17 |
| Contract and Collaborative Research: | |
| Intrapartum Fetal Monitoring | EB-19 |
| Proposed Analysis of a Major Cause of the Sudden Infant Death Syndrome (SIDS) | EB-21 |
| Development of Animal Model for Studies of Sudden Infant Death Syndrome (SIDS) | EB-22 |
| Sudden Death in Cats: A Spontaneously Occuring Model for Sudden Infant Death Syndrome (SIDS) in Man | EB-23 |
| A Study of Monozygous and Dizygous Twins to Ascertain Possible Neurophysiologic Factors for the Sudden Infant Death Syndrome | EB-24 |
| Development of Sleep and Cardiopulmonary Regulations within Sleep: Clinical Studies of a Functional Mechanism for Risk of Sudden Infant Death | EB-25 |
| Analysis of State Integration and Related Cardiopulmonary Functions in Infants at High and Low Risk for the Sudden Infant Death Syndrome | EB-27 |
| <u>Adult Development and Aging Branch</u> | |
| Summary Report | EC-1 |
| Mission | EC-1 |
| Growth of Extramural Research on Aging | EC-1 |
| Research and Training Programs | EC-1 |
| Research Activities | EC-2 |
| Training Grants | EC-7 |
| Contract and Collaborative Research: | |
| Contract to Breed, Rear and Maintain a Colony of Inbred Aging Laboratory Rats for Aging Research (Modified) | EC-15 |
| Development of a Production Colony of Three Genotypes and a Non-inbred Strain of Laboratory Mouse for Aging Research | EC-17 |
| Production Colony of Aging Rats in an Isolator Environment | EC-19 |
| Summer Course in the Pathobiology of Aging | EC-21 |
| Quantitative Studies of Aging Human Diploid Fibroblasts <u>in vitro</u> | EC-22 |
| Production, Characterization and Distribution of Human Diploid Cell Strains | EC-23 |

| | <u>Page</u> |
|--|-------------|
| Prevention of Bone Loss in the Menopause | EC-24 |
| Origin and Action of Estrogen in the Postmenopausal Woman | EC-25 |
| A Retrospective Study of Postmenopausal Women With and Without Estrogen Replacement Therapy | EC-27 |
| Analysis of Aging-associated Involution of Cellular Immunity in Mice | EC-28 |
| Longitudinal Interstudy Program | EC-30 |
| Review, Analysis, and Evaluation of Models for Forecasting Future U.S. Population | EC-31 |
| Summer Course on Psychological and Social Aspects of Aging | EC-32 |

Mental Retardation Branch

| | |
|--|------|
| Summary Report | ED-1 |
| Mental Retardation Research Centers | ED-3 |
| Research Activities | ED-4 |
| Genetics and Inborn Errors of Metabolism | ED-4 |
| Teratology | ED-5 |
| Early Intervention | ED-6 |
| Malnutrition and Learning | ED-6 |
| Amelioration and Treatment | ED-6 |

Collaborative Research and Contracts:

| | |
|---|-------|
| Diagnostic and Intervention Studies of High-risk Infants | ED-9 |
| Definition of a Behavioral Phenotype in the Cornelia deLange Syndrome | ED-11 |
| An Automated System for Chromosome Analysis | ED-13 |
| Intellectual and Behavioral Consequences of Severe Malnutrition in Infancy: A Collaborative Study | ED-15 |
| Prevention of Prematurity and Developmental Disorders Through Nutritional Supplementation and Laboratory Studies on Prenatal Malnutrition | ED-16 |
| An Investigation of Certain Relationships Between Hearing Impairment and Language Disability | ED-18 |
| The Development of Materials for Application of Behavior Modification Skills by Parents of Mentally Retarded Children | ED-21 |
| Coordination of Regional Cytogenetic Registries | ED-22 |
| Social Aspects of Mental Retardation in Poland | ED-24 |
| Follow-up Study on the Application of Behavioral Modification Skills by Parents of Mentally Retarded Children | ED-25 |

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Office of the Director

In fiscal year 1974 the following significant events directly affected the Institute's program and administrative activities:

- the National Institute on Aging was established;
- in recognition of the need for more research, counseling, and information collection and dissemination on the Sudden Infant Death Syndrome, Congress passed Public Law 93-270. The National Institute of Child Health and Human Development has been designated the lead agency in the implementation of this Act;
- continued restrictions on employment compounded by requirements to reduce average grades;
- significant increases in dollars (\$32,483,000), primarily for Population Research and Child Health Research;
- establishment of a career development training program for NICHD employees who are presently in dead-ended positions.

Budget

The FY 74 operating level for the Institute increased substantially from last year due to an increased appropriation to \$124.9 million and the release of \$19 million in FY 73 impounded funds. This increase resulted in expansion as indicated below, in all the mechanisms of research support and in all the program areas of the Institute.

NICHD - Comparison by Mechanisms (dollars in thousands)

| | <u>1973</u> | <u>1974</u> | <u>Increase</u> |
|---|----------------|----------------|-----------------|
| Research Grants | \$62,150 | \$83,476 | \$+21,326 |
| Fellowships | 3,066 | 6,048 | +2,982 |
| Training Grants | 6,905 | 11,017 | +4,112 |
| Intramural, Laboratory & Clinical Research | 10,264 | 11,317 | +1,053 |
| Research & Development Contracts | 18,996 | 20,737 | +1,741 |
| Research Management and Program Services | 5,881 | 6,603 | +722 |
| Management Fund | 4,155 | 4,702 | +547 |
| Total | <u>111,417</u> | <u>143,900</u> | <u>+32,483</u> |

NICHD - Comparison by Programs (dollars in thousands)

| | | | |
|---|----------------|----------------|----------------|
| Population | \$39,905 | \$51,187 | \$+11,282 |
| Child Health | 53,225 | 68,038 | +14,813 |
| Perinatal Biology & Infant Mortality | (16,212) | (23,174) | (+6,962) |
| Growth and Development | (17,774) | (23,561) | (+5,787) |
| Mental Retardation | (19,239) | (21,303) | (+2,064) |
| Aging | 12,680 | 17,023 | +4,343 |
| Other Management Fund | 4,155 | 4,702 | +547 |
| General Research Support Grants | 1,452 | 2,950 | +1,498 |
| Total | <u>111,417</u> | <u>143,900</u> | <u>+32,483</u> |

Staffing

At the beginning of the fiscal year the Institute developed a staffing plan based on a ceiling of 525 positions. During the year this was reduced to 514 and then restored to 523. The following table compares the staffing patterns of FY 73 and 74:

| | <u>Actual 6/30/73</u> | | | <u>6/30/74 Estimate</u> | | |
|-------|-----------------------|--------------|---------------------|-------------------------|--------------|---------------------|
| | <u>Full-time</u> | <u>Other</u> | <u>Non-ceiling*</u> | <u>Full-time</u> | <u>Other</u> | <u>Non-ceiling*</u> |
| OD | 48 | 12 | 8 | 41 | 9 | 2 |
| OSD | 315 | 32 | 127 | 330 | 29 | 165 |
| PS | 68 | 9 | 3 | 65 | 9 | 3 |
| EP | 38 | 8 | 2 | 36 | 8 | 1 |
| CPR | 36 | 1 | 1 | 36 | 1 | |
| EB | 16 | 4 | 5 | 15 | 4 | 4 |
| Total | 521 | 66 | 146 | 523 | 60 | 175 |

*Includes Visiting Fellows, Guest Workers, Guest Scientists, ACCESS, Junior Fellows, Management Interns, Stay-in-School.

Grade De-escalation

Three years ago the Federal Government initiated a program to reduce and control the average grade of its employees. The Institute met its average grade target last year and anticipates no problem in doing the same for FY 74.

Average NICHD Grade Targets

| | |
|---------------|---------|
| June 30, 1971 | 9.0773* |
| June 30, 1972 | 8.8080* |
| June 30, 1973 | 8.0703 |
| June 30, 1974 | 8.1947 |

*Excludes summer employment - summer employment included at NIH level for these years

Legislation

On April 22, 1974, the President signed into law the Sudden Infant Death Syndrome Act (Public Law 93-270) concerning research, counseling, and information collection and dissemination related to SIDS. While not specifically authorizing funds for these purposes, PL 93-270 amends the PHS Act to include SIDS as part of the Institute's research mandate and requires the Secretary to report to four Congressional Committees the estimated NIH expenditures for NIH research efforts on SIDS.

In May of 1974 the President signed into law S. 775 establishing a National Institute on Aging within the NIH to study the aging process and the health problems of the aged. This law adds sections 461 through 466 to the PHS Act establishing the NIA for the conduct and support of biomedical, social, and behavioral research and training related to the aging process and the diseases and other special problems and needs of the aged. The Secretary is specifically directed to establish a National Advisory Council on Aging, to carry out public information and education programs relating to aging, and to develop a plan to promote and coordinate aging research.

Equal Employment Opportunity

During FY 74 the NICHD staff has been involved in the initiation of two especially significant projects. One has been the preparation of a training program designed for individuals who are dead-ended in, or desire to change, their present classification series. The training program will operate in accordance with policies established by the NIH Merit Program and the NIH Upward Mobility Training Agreement and will function as a supplement to help identify, train and place qualifying employees for the purpose of fostering their career development. The plan currently calls for three individuals to enter the program annually. The losing office will hire a permanent, full-time replacement while the trainee embarks on a training assignment carefully selected to meet the needs of the Institute and the aptitude, interests and background of the trainee.

The EEO Committee and management officials have also been working to establish an EEO Committee at the Gerontology Research Center to provide an effective advisory body for those employees geographically isolated from the main NIH campus. The formation of such a committee is also expected to strengthen existing NICHD efforts in the area of equal employment opportunity, especially as it relates to planning and implementing an appropriate affirmative action plan. Specifically, the GRC EEO Committee will act as an advisory body to the Institute Director, through the NICHD EEO Committee, and will assist in the identification and solution of areas of concern within GRC.

Manpower Management

In accordance with the NIH Manpower Management Program, the Office of Administrative Management staff conducted or participated in four management surveys in FY 1974. Approximately 114 full-time permanent positions were

reviewed. The Office of Management Policy, NIH, conducted a Functional Review of the Program Statistics and Analysis Branch; the survey's conclusions and recommendations will be analyzed and, where pertinent, will be implemented in FY 1975.

Office of Research Reporting

FY 74 saw major changes in this office resulting primarily from a DHEW public affairs cutback and reorganization. These changes included the renaming of the Office of Public Information as the Office of Research Reporting (ORR), the transfer of the one professional position located at the Gerontology Research Center from NICHD to the Office of the Director of NIH, and the transfer of "public affairs" activities from ORR to the Office of the Associate Director for Communications, NIH. At the end of FY 73, the Scientific Publications Section was abolished and a position was created for a scientific publications officer.

The largest single project undertaken this year has been the creation and distribution of three publications in a nationwide public education campaign designed to decrease the incidence of mongolism. Other activities included the answering of approximately 8,000 public inquiries and the distribution of over 100,000 publications, the answering of 180 Congressional and Presidential inquiries, participating in the preparation of materials for the Congressional appropriations hearing, the conducting of a series of very successful population seminars for science writers, and health education projects relating to the Sudden Infant Death Syndrome, the first three days of life, low birth weight, and the menopause.

Office of Program Planning and Evaluation

The mission of this office is to develop the information needed for the planning and formulation of the Institute's major goals and the means for appraising the progress toward these objectives. In FY 74, OPPE has been involved in the following activities: preparing reports in reply to congressional, departmental and interagency inquiries; keeping Institute personnel informed about pertinent legislation; participating as part of the team effort in the fight against the Sudden Infant Death Syndrome; working in the area of pharmacological research concerned with the effects of medications on fetal health; participating in the development of plans for a clinical research unit in reproductive research and perinatal biology; preparing a report on a research conference in the area of man-made environment and child development; and maintaining liaison with agencies whose missions are complementary to, or supportive of, that of the Institute.

Office of the Associate Director for Epidemiology and Biometry

In the past year the research and consultation activities of this office have been somewhat hampered, both quantitatively and qualitatively, by the lack of adequate staffing. The progress of such activities in the two branches (described below) has, nevertheless, been very good. The list of accomplishments for FY 74 includes the preparation of twenty current and future publications, serving as project officers on a number of contracts, conducting several projects, and giving talks before various professional and university groups. In addition, the Associate Director sat on four committees outside of NICHD, presented three talks and was appointed a Johns Hopkins University Centennial Scholar.

Epidemiology Branch

This branch performs epidemiologic studies in areas related to the mission of the Institute and provides epidemiologic consultations to scientists in both the intra- and extramural programs. FY 74 has seen a concentration on the etiology of and prevention of prematurity and infant mortality. In addition, a considerable amount of staff time has been spent on a variety of "non-project" activities. A full accounting of all activities can be found in the body of the Annual Report.

Biometry Branch

The activities of this branch in FY 74 have been divided primarily among three areas. One of these areas has been to function as a national data center for studies concerning the risks associated with amniocentesis and the use of phototherapy as a treatment for bilirubinemia. The second such area has been to conduct research and, as an extension of this research, to initiate contracts. Such research projects have included planning longitudinal studies for the Gerontology Research Center, studying the affects of oral contraceptives, preparing a monograph comparing the curettage and suction methods of abortion, monitoring a variety of diagnoses in the newborn, and publishing in the fields of congenital malformations, the Sudden Infant Death Syndrome, and birth weight. The third area of concentration has been statistical consultation to professional personnel in the Institute involved in a wide variety of research projects.

Office of the Associate Director of Program Services

This past year there was a significant increase in the workload of this Office accompanied by a reduction of six full-time permanent positions distributed between the two component branches--Program Statistics and Analysis, and Grants and Contracts Management (both described below).

The Conference Assistant Position was abolished and the functions divided between the conference program sponsors and the Administrative Officer. The Committee Management Assistant continued to provide services to the nine advisory committees of the Institute. The National Advisory Council held three meetings in FY 74 with only eight appointed members of a total authorized membership of fourteen.

Grants and Contracts

During FY 74 the Institute supported approximately 1,732 research grants, training grants, fellowships, new research training fellowships, research career awards, research career development awards, and research contracts for approximately \$118.4 million (including released funds impounded in FY 73).

Summary of Institute Supported Projects (% of total FY 74 committed)

| <u>Category</u> | <u>% of the number of projects funded</u> | <u>% of the dollars funded</u> |
|--|---|--------------------------------|
| Research grants | 65.3 | 69.9 |
| Training grants | 5.7 | 10.5 |
| Fellowships | 2.3 | 0.4 |
| New research training fellowships | 7.8 | 1.5 |
| Research career & research career development awards | 5.3 | 1.9 |
| Contracts | 13.5 | 17.5 |

The analysis in the body of the Annual Report gives an accounting of the number of projects funded and the amount of funding for each category, a comparison with FY 73, and the system used to assign funding priorities.

Program Statistics and Analysis Branch

Activities in the Program Analysis Section for FY 74 remained unchanged in content but expanded in quantity. Basically these activities included the assignment of NICHD applications to the various programs within the Institute, the classification of grants, and the preparation of numerous reports, both recurring and requested.

The Statistical Analysis Section participated in the advanced planning and preliminary work for the contemplated switchover to machine input drawn from

the Division of Research Grants IMPAC system. In addition, the section remained responsible for preparing no less than thirteen different recurring reports and for furnishing information to Congress, other institutes and the Ford Foundation.

The major emphasis of the Information Systems Section has been on the planning, designing and programming for input linkage between the IMPAC system and the PSAB machine data system. This is in addition to improving the master file update system and handling the computer aspects of, and the publication preparation for, several publications.

Center for Population Research, Office of the Director

Organized six years ago and consisting of four branches (described below), the CPR is currently operating under a stringent personnel ceiling that has become even more critical in FY 74. Regardless, a high level of quality activities has been maintained as evidenced by the following accomplishments: production of a monthly bibliographic journal dealing with biomedical research in the population field, six publications based upon Institute sponsored conferences and workshops, fourteen lectures presented by staff, fourteen published articles written by staff, eight honors received by staff, occasional monographs published as individual books on a non-periodic basis, an annual report evaluating current federal research efforts with recommendations, an annual updating of its section of the Inventory of Federal Population Research, and staff provision to coordinate population research activities among various federal agencies.

Behavioral Science Branch

The research activities supported by this branch investigated the ways in which individuals are motivated to reduce family size and the interrelationships between population size, growth, and distribution and economic, social, and psychological factors. These activities take into account individual, familial, institutional, and social processes of great complexity and make use of a multi-disciplinary approach consisting of sociology, psychology, economics, anthropology, medicine, and statistics.

In FY 74 the particular areas of focus examined the determinants of and regulation of fertility, and the consequences of population growth and change as a guide to the formulation of public policy. Toward the achievement of these goals there were sixty-two active contracts (twenty-one of which were extensions of projects negotiated in previous years) studying the socio-economic correlates of fertility; migration, social mobility and residence patterns; the sex roles and socio-psychological correlates of fertility; the demographic and attitudinal factors affecting family formation; the consequences of population growth; the possible development of population policies; and fertility, abortion, illegitimacy and family planning.

Contraceptive Development Branch

The mission of this branch is to develop an array of safe, effective and reversible methods of contraception for both men and women. The wide range of research activities from clinical trials of drugs and devices to chemical modifications of existing drugs to fundamental research in the reproductive systems of both sexes--has been supported through 128 contracts.

Trends of the past year have shifted toward the development of new drugs and new contraceptive measures for men. Funds for these purposes have been channeled into two broad areas of endeavor--directed fundamental (biomedical) research and product development. Projects under study in the former area are the detection of ovulation, the ovum and oviduct, the cervix, the pituitary-ovary-corpora luteum system, the prostoglandins, the male reproductive processes, and the mechanism of hormone action. Projects falling into the latter program area include new drug development and testing, drug delivery systems, male and female sterilization techniques, and male and female clinical studies. In addition, the branch is supplying the scientific community with hormone samples produced through research contracts.

Population and Reproduction Grants Branch

This branch provides support for fundamental biological and chemical research and training in biomedical disciplines dealing with the determinants and consequences of population phenomena. Funds are used to support Population Research Centers, Program Project Grants, the annual meeting of the directors of the PRCs, the Research Career Program, fellowship programs, training programs, staff, symposia, and workshops.

The scope of PRGB supported biomedical and behavioral-social research is so large and so broad that it would be futile to try to capsule it in so short a summary. The interested reader is therefore directed to the body of the Annual Report for a meaningful review.

Fertility Regulating Methods Evaluation Branch

Despite a serious shortage of professional personnel, the following programs have been pursued in FY 74: the continuation of the Walnut Creek Contraceptive Drug Study with emphasis on the clinical effects; a study of the relationship between oral contraceptive use and breast cancer and cervical dysplasia; a study of the relationship between oral contraceptive use and thrombosis and thromboembolism; the characterization of the biochemical aspects of hypertension among users of oral contraceptives and Premarin therapy; the metabolism of and the metabolic effects of oral contraceptive steroids; the assimilation of information on the possible long-term medical consequences of vasectomy; and a survey of the nutritive health status of oral contraceptive users.

Office of the Associate Director for Extramural Programs

The extramural area consists of four branches each of which, for the first time this past year, has had its own budget for direct operations. This change has been so successful that all of the Program Directors encourage the Institute to extend this concept to grants and contracts.

During FY 74 the Institute research review committees met a total of ten times and concentrated on program projects and center grants. The NICHD Council has reviewed in depth all of the Extramural Programs and has encouraged the expansion of research support bearing on the Sudden Infant Death Syndrome and the aging processes. Council has also revised its scientific review provision within NICHD so as to require a full scientific evaluation of program projects and centers during the fourth year of a five-year award rather than every third year.

Growth and Development Branch

As has been the case in past years, this branch has supported approximately 25% of the NICHD research grants during FY 74. An area of high priority this year has been studies relating to adolescence. All members of the staff have been involved in this endeavor and three conferences have been held, but expenditures remain modest. Other research activities supported include mechanisms of biological growth; immunological and pharmacological studies of development; developmental behavioral biology; learning and cognitive development; human communication, personality, and social development; nutrition; and physical growth. Additional activities included a major publication, a renewal of research training activities and three scientific conferences.

Perinatal Birth and Infant Mortality Branch

The mission of this branch is to support and promote a coordinated program of research and training which will increase the understanding and development of knowledge as it relates to pregnancy and maternal health, embryonic development, fetal growth, and infant well-being through the first year of life. PBIM administers approximately 21% of the Institute's extramural activities and consists of four broad program areas. The Pregnancy and Maternal Health Program supports a wide variety of studies pertaining to the physiological factors which influence the gravida state during the antepartum, intrapartum, and puerperium. Specific areas of accomplishment this past year have included, but are not limited to, the influence of estrogen and progesterone, the origin of X-cells and the hormonal stimulation of the onset of labor.

The Developmental Biology Program supports research investigating the developmental aspects of prenatal and neonatal health problems. The subject areas involved cover a wide spectrum including the serum folate, polypeptide chain termination, and the level of arylsulfatase associated with matachromatic leukodystrophy.

The Fetal Health and Development Program is concerned with basic problems related to development at the tissue and organ levels. Specific areas of

investigation this year included studying the dependency of fetal growth and development on an adequate oxygen supply, the interrelationship between nutritional and genetic factors during pregnancy and development, chromosomal aspects of myeloproliferative disorders, and D-trisomy mosaicism and neurological defects.

The Infant Survival and Well Being Program supports research investigating the postnatal period from birth to one year of age. Areas of special interest included low birth weight and death in newborns associated with faulty adaptation to the extrauterine environment.

Additional activities of this branch for FY 74 included seven workshops and conferences, twelve publications, and a continuation of the Institute's efforts to expand the Sudden Infant Death Syndrome program.

Adult Development and Aging Branch

The mission of this branch is to support research into the biological, medical, and behavioral aspects of aging. The branch sponsors, in conjunction with the Veterans Administration, a series of yearly meetings to coordinate certain longitudinal studies on aging. Also, NICHD has the authority to initiate center grants to institution for the purpose of expanding and improving research programs concerning the aging processes.

A list of research activities supported in FY 74 includes the following topics: organismic models of aging, aging of rapidly dividing diploid cells, endocrine changes with aging, connective tissue aging, immunological changes with aging, neurological changes with aging, cognitive changes with aging, societal aspects of aging, other biological aspects of aging, and disease and psychologically oriented research.

Mental Retardation

This branch is assigned the task of developing and supporting research aimed at the prevention and amelioration of mental retardation. The 247 discrete projects supported in FY 74 covered virtually every known dimension of mental retardation research. The development of the Mental Retardation Research Center program represents a potentially unique resource for multidisciplinary and collaborative research between biomedical and behavioral scientists in an attempt to accomplish the branch mission. More than six hundred investigators from a broad range of basic, clinical and applied sciences are involved in this effort. These centers have been well served by the Institute's development of innovative policies of fiscal support.

Additional support has been directed toward the following target areas: lowering the incidence of mental retardation associated with genetic and inborn metabolic errors; gathering evidence concerning the effectiveness of early intervention for improving the development of the retarded child; investigating the possible relationship between malnutrition and impaired growth and intellectual development; working to develop alternatives to the present system of residential care in the US; attempting to effect behavior modification in the home setting; gathering data on the relationship between maternal behavior and the fostering of optimal development; and mounting a broad research attack in all related areas.

Office of the Scientific Director

This office consists of seven branches and laboratories and the Gerontology Research Center, and constitutes the Intramural Program of NICHD. Research accomplishments (summarized below and detailed in the body of the Annual Report) have continued to emanate from the fields of aging, reproduction, and maternal and child health. New facilities are in various stages of being planned, designed, and constructed with the purpose of fostering these research programs.

In FY 74, 117 visiting scientists worked in Institute laboratories in addition to the 332 budgeted and 58 "other" positions participating in the intramural research programs.

Developmental Immunology Branch

The center of focus for this branch in FY 74 has been a series of studies involving Haemophilus influenzae type b. These studies included the immunogenicity and protective effect of the capsular polysaccharides of the virus, the heteroimmunogenicity of cross-reacting bacteria to the virus, and the structural basis for the cross reactions observed between the virus and enteric bacteria.

Other activities included a symposium on the topic Pneumocystis carinii Pneumonitis and research projects studying the potential toxicity of cross-reacting bacteria to newborns, the plasmid directed E. Coli enterotoxin, the structure and function of the pneumococcal C-polysaccharide, and the primary structure of streptococcal proteinase.

Social and Behavioral Sciences Branch

Research for FY 74 has been concerned largely with various aspects of early cognitive, motivational, and social development. One such aspect under investigation is that of early environmental influences. Studies in this area have focused upon broadening the scope of our knowledge relating to the differentiation of the early environment; the role and influence of the father during early development vis-a-vis his relationship with the son, the daughter, and the wife; the motivation of the infant to master his environment; and the potentially adverse environmental circumstances associated with prematurity.

Other aspects under scrutiny included the development of attachment, the self-image of the mother, the role of early infant temperament in eliciting stimulation and influencing his experiences, the fear of strangers, and the relationships between analgesia and anesthesia administered during delivery and early infant temperament.

Reproduction Research Branch

The mission of this branch is the development of scientific data and theory basic to reproductive biology. A multi-disciplinary approach has been employed utilizing physical chemistry, biochemistry, mathematics, reproductive biology, endocrinology and internal medicine.

Areas of investigation this year have included the protein hormones, the solubilized gonadotropin receptor of the testis, the biochemistry of the gonadotropins, follicular growth, and the cell of origin of factor(s) regulating FSH secretion. Clinical investigations have included amenorrhea, HCG in the fluids of patients with molar pregnancy, emn with idiopathic oligospermia, and the relationship of renin activity to strokes and heart attacks.

Behavioral Biology Branch

Research activities in this branch are divided among three sections. The Section Neurobiology has been involved in many studies including, but not limited to, the growth of neurons in tissue culture, the synaptic interactions of spinal cord cells and dorsal root ganglion cells, the activity of CAT in the interactions between spinal cord cells and muscle cells, the CAT activity of pineal organs, and the stimulation of CAMP accumulation in brain and glial tumor cells.

The Section on Functional Neurochemistry has conducted extensive research into the correlation between protein synthesis patterns and specific cell functions in neurons and into the establishment of the molluscan nervous system as a model for the excitation-metabolic coupling and the hypothalamic regulatory systems in mammals.

The Section on Brain and Behavior has continued its efforts to develop and exploit behavioral techniques as optimizing methods for food reward training in discrimination studies in monkeys. Initiated in FY 74 has been a study to investigate the neurophysiological and functional properties of a major pathway from the cortex into the temporal lobe and an extensive quantitative analysis of the variability in production of Isolation Peeps by captive monkeys.

Laboratory of Molecular Genetics

This laboratory is currently composed of nine research groups with the overall objective of enhancing our understanding of the molecular processes involved in the transmission of genetic information and in the expression of genetic information during growth and differentiation. These studies require a multi-disciplinary approach consisting of genetics, biochemistry, immunology, virology and cellular biology.

Much insight is being gained through experimentation involving the use of viruses. Studies this past year concentrated on the actions of bacteriophage, the replication of viral genes, the process of viral assembly, the identification of viral macromolecules, and the identification and localization of specific viral gene functions.

Other areas of investigation included the role of nucleotides in macromolecule synthesis, the biosynthesis and accumulation of mRNA, the differentiation of chick embryo lens fiber, the cellular regulatory pathways affecting antibody synthesis and the regulation of the expression of mammalian genes.

Laboratory of Biomedical Sciences

Research activities in this laboratory are divided among four sections. Interests in the Section on Developmental Enzymology center on the purification and characterization of the enzymes associated with the fetus, placenta and maternal plasma. Studies this year have concerned the enzymes involved in the metabolism of lysine, glycogen, catecholamines and oxytocin.

Scientists in the Section on Intermediary Metabolism have been investigating the basic biochemistry of the brain and the conditions which alter normal brain functions. Specific efforts have focused upon the development of laboratory analogs of mental retardation, the investigation of the biosynthesis and degradation of macromolecules in neural tissue, and the elucidation of the mechanisms of transport of small molecules into and out of the brain.

The Section on Physiological Controls studies basic endocrine and neuroendocrine mechanisms and factors controlling their development. Specific areas studied this year have been the mode of action of cAMP and the regulation of glycogen cycle enzymes.

The Section on Developmental Pharmacology has concentrated on the effects of polycyclic hydrocarbons and insecticides, and on the subcellular processes involved in the induction by drugs of pharmacologically important enzymes. The combination of these environmental stimuli and the host's genetic constitution can affect fetal growth, development and health.

Pregnancy Research Branch

This branch was considerably strengthened in FY 74 by the addition of more professionals and a new animal operating suite. Also, occupancy of the clinical facility is expected to occur this year and efforts are now being made toward the staffing of this facility.

The two main areas of research have been endocrine physiology and the biochemistry of steroid hormones during pregnancy. Data obtained from animal models (Rhesus monkeys, baboons, and Dorset sheep) have strengthened the belief that extrapolation from these models to human pregnancy is a valid approach in the investigation of these subjects.

The Gerontology Research Center

Due to its isolation from the NIH campus, the GRC is forced to maintain a high degree of self-sufficiency in supporting its research programs. These services include the Technical Development Section (to design, construct, maintain, and service scientific equipment), on-line computer capabilities, the Photography and Arts Section, the Animal Resources Facility, the Public Information Office, and a library staff.

The Center also sponsors a program which, during the past year, supported eighteen guest scientist programs staffed by twenty-seven investigators and twenty-one supporting personnel. The areas of study included endocrinology, age differences in cardiac performance in the rat, cell biology and gastrointestinal physiology.

Laboratory of Behavioral Sciences

This lab has continued its commitment to train scientists in aging research as well as to conduct original research. Thus the staff includes undergraduate and graduate students in psychology, post-doctoral fellows and members with university appointments.

Research in man has included investigations into the changes in cognitive and perceptual functions occurring with age, the ability to train patients to control their systolic blood pressure, and the training of patients with chronic fecal incompetence to become continent.

Other areas of study have included the basic mechanism of memory and problem solving, the autonomic mechanism of learned voluntary control of heart rate in the rhesus monkey, and other varied programs being carried out with lower animals.

Clinical Physiology Branch

FY 74 marks the end of the seventh two-year cycle of tests in the Longitudinal Study of Human Aging and has been chosen as the appropriate time for a comprehensive summarization of our longitudinal data. Summaries of these computer-based data have been completed for twenty-one variables and have been reviewed for accuracy and completeness. Many of these variables will be continued into subsequent test cycles while others will be dropped or replaced.

Research efforts have been extensive and have included tapping test and activity questionnaire analysis, Vitamin B6 intake studies, airway obstruction pathophysiology, renal response to water deprivation, the use of antipyrine to estimate total body water, various dermatoglyphic studies, the affect of the X_g blood group on mother-fetus incompatibility, the improvement of routine urinalysis as a screening technique for renal diseases, amino acid metabolism studies, investigations into hemochromatosis and Fabry's Disease, studies of hormone differences between young and old subjects, and characterization of the chemistry of renin.

Laboratory of Cellular and Comparative Physiology

Research activities have continued along the lines established last year with particular emphasis directed toward an understanding of how aging affects the proliferation and differentiation processes at the cellular and molecular levels. Associated with these goals, there has been a change in the research approach to understand biologic mechanisms at the expense of biologic phenomena.

The focus of research in this context has been the immune system. Particular emphasis was placed upon, but not limited to, the cellular and functional properties of subpopulations of lymphoid cells; the characterization of artificially induced and naturally occurring immunodeficiency states; and the effects of aging on the modulation of proliferation and differentiation of immunocompetent cells.

Laboratory of Molecular Aging

Two of the major research programs pursued in this laboratory in the past year have concentrated on the control mechanisms by which metabolic processes are regulated (with special emphasis on membrane and mitochondrial biology) and on the interaction of the molecules that are involved in the propagation of genetic information.

Other projects included studying the effect of cell age on the concentration of certain metal ions, the decreased ability of aged cells to actively protect themselves, the ability of vinyl analogs of polynucleotides to produce selective toxicity against viral leukemia, and the ability of hemoglobin-bound copper to oxidize the iron in the hemoglobin in the absence of oxygen.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Office of Research Reporting (ORR)

Organization and Personnel

The most important influence on this office during fiscal year 1974 was the DHEW public affairs cutback. Though the function of this office was not considered to be public affairs by the Secretary's definition, the Institute's public information program was affected in the following ways:

1. This office, formerly called Office of Public Information (OPI), was renamed the Office of Research Reporting (ORR).
2. One professional NICHD slot was transferred to the Office of the Director, NIH. Though basically carrying out the same responsibilities as before the reorganization and still working out of the Gerontology Research Center (GRC), the information person filling this position is now responsible to the Associate Director for Communications, NIH.
3. Those activities deemed public affairs are now handled by a representative of the Associate Director for Communications, NIH. The change was instituted to implement the Secretary's directive that Institute program persons be more directly accessible to the press. Thus, some activities once initiated and carried out by this office are now suggested and supported by this office. Functions other than public affairs activities, including freedom of information, clearing scientific publications and speeches, reporting to Congress, internal reporting, responding to public inquiries and producing scientific and health education materials remain under ORR auspices.
4. Due to this Agency-wide information reorganization, a large amount of staff time went into supplying reports and information required by the Secretary. For example, several reports of publications costs, past, present and projected, have been required, though the issue of whether publications activities will be basically changed has not been resolved.

In addition to the preceding changes, the ORR lost one professional slot over and above the three slots lost last fiscal year. At the end of fiscal year 1973, the Scientific Publications Section was abolished and a position for a scientific publications officer was established to advise on and coordinate the Institute's scientific publications activities. This officer is clearance officer as well as technical editor for the Institute's scientific books.

Activities at the Gerontology Research Center

Although this position has been relocated as described earlier, the functions of the person filling this slot remain basically the same. The most significant change in GRC information activities this year was the 62 percent rise in the number of public information inquiries handled. The number of people

visiting the Baltimore facility for tours and background briefings rose 14 percent over last year's count. Visitors included gerontology trainees, nursing students, senior citizens groups, high school and college classes, and foreign scientists.

Although fewer inquiries were made by the news media, results of media contacts reached greater audiences than in the past. For example, footage shot for an aging documentary by NBC-TV News will be viewed by an audience of millions. Print media coverage of the GRC included articles in the National Observer, Newark Star-Ledger, Baltimore Sun magazine, Medical World News, Readers' Digest, and Prevention Magazine. Further press activity was handled through a full working press room managed by the GRC Public Information Officer at the annual meeting of the Gerontological Society.

In addition, the GRC Library continued to serve as the foremost regional source of worldwide literature in the aging field. This year, the library staff indexed 3,109 titles for inclusion in the periodical listing "Current Publications in Gerontology and Geriatrics," published in the Journal of Gerontology.

Freedom of Information

The chief of this office is considered Freedom of Information Officer for the Institute and hence advises on and coordinates Institute responses and requests covered by the Freedom of Information Act. The current trend is toward more requests and more liberal interpretation of what information is properly considered within the public domain.

Mongolism Education Campaign

Major emphasis for this year has been on the nationwide public education campaign to decrease the incidence of mongolism. This large-scale project is a departure from Institute information activities heretofore. Through the use of public relations, publication preparation and dissemination, audio-visual activities, public service announcements, seminars and other scientific and professional education, the campaign seeks to inform women over age 35 of their increased risks of giving birth to Down's syndrome children and to advise them of options open to them. The campaign has a physician and professional education component that supplements the public information activities. In connection with the education campaign, three publications were prepared this year: Antenatal Diagnosis and Down's Syndrome, What is Mongolism?, and Facts About Mongolism for Women Over 35.

Other health education projects were undertaken in the areas of the sudden infant death syndrome, the first three days of life, low birth weight, and the menopause.

Public Inquiries

This year, the ORR answered about 8,000 public inquiries and distributed some 108,200 publications in response to these requests. The usual summer slump, believed to follow closing of schools for vacations, was not evident, and

public inquiries and requests came in steadily throughout the year. In addition to the continued high interest in the sudden infant death syndrome and population, this year the public asked for a great deal of information on the menopause, learning disabilities and mental retardation. An NIH Information Index, compiled through the aid of each NIH information office, is now in use as an assist in the routing of inquiries to the most appropriate and responsive sources. Periodically revised and updated, the Index is improving handling of incoming inquiries.

Congressional and White House Activities

In addition to handling approximately 90 Congressional and 90 Presidential inquiries and requests (a number about equal to last year's), this office also prepared materials for Congressional appropriations hearings. This included drafting the Director's Opening Statement and preparing highlights of research progress (this year used for background information) and special reports on aging research, mental retardation, communications, family planning, and the sudden infant death syndrome.

Science Writers' Seminars

Following a series of very successful seminars for science writers set up and handled by ORR in the area of population research, the NIH information network has decided to emulate the NICHD example and see that similar seminars are done more frequently at NIH and through central leadership.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Office of Program Planning and Evaluation

The Associate Director for Program Planning and Evaluation and his staff are responsible for developing the information needed for the planning and the formulation of the Institute's major goals and objectives and the means for appraising progress toward these objectives.

In performing these functions, the Office: (1) sponsors and conducts studies on the nature of the emerging problems and needs within the broad field of child health and human development; (2) stimulates and provides guidance for the analysis of the status, character, and extent of scientific activity in problem areas and the resources needed for further Institute efforts; (3) coordinates activities leading to the selection of Institute objectives and the development of program plans; (4) prepares and maintains a comprehensive multi-year program plan, utilizing operational planning of program activities to assess progress toward objectives and to evaluate accomplishments, and (6) maintains an awareness of ongoing Congressional activities, keeps staff informed of legislative developments, and coordinates matters of mutual concern with other interested governmental and non-governmental organizations.

Dr. S. W. Greenhouse is Acting Associate Director of the Office of Program Planning and Evaluation (OPPE) of the NICHD. His staff includes an Assistant Director for Clinical Programs, Facilities and Resources; an Assistant Director for the Behavioral Sciences; an Assistant Director for the Biological Sciences; a Medical Officer (Pediatrician) currently on a consultant basis; a Program Analyst (evaluation and legislative liaison); a Legislative Assistant; and supporting secretarial staff.

In Fiscal Year 1974 the OPPE prepared several reports on aspects of the research programs of the Institute in response to requests from Congressional Committees and Departmental offices. Similar material was developed for interagency coordination purposes. There continues to be much interest in how Institute research programs pertaining to early child development interface with other Federal efforts in this area. Similar work on training programs were involved during FY 1974 despite the fact that they are being phased out. Several pieces of legislation concerned with Federal support of research and services specifically aimed at improving the health status and learning capabilities of children have been introduced in the Congress. All of these bills have had some impact on NICHD research and program activities in FY 1974. The Office keeps all personnel informed about this legislation. Staff of OPPE, in cooperation with the Budget Officer, Executive Officer, and Program Personnel of the Institute, developed the Forward Plan for FY 1976-80 and the Operational Plan for FY 1974.

The death of infants suddenly from unknown cause accounts for about one tenth of the total infant mortality in the United States. OPPE staff

continues to cooperate in Extramural Program efforts by the Perinatal Biology and Infant Mortality Branch (PBIM) to identify causes of the sudden infant death syndrome (SIDS) and to effect its prevention. These efforts also include staff of the Epidemiology and Biometry Branches of the Office of Epidemiology and Biometry. This team approach to an important pediatric problem includes development of collaborative research projects and the definition of research goals and needs as well as the coordination of Institute research efforts in this area. The representative of Biological Sciences in OPPE manages the review of research contract proposals concerned with SIDS. He also collaborates with the Program Director, PBIM in the development of program plans in this and other priority areas concerned with the prevention of infant morbidity and mortality. Plans are developed in accord with the principles of management by objectives and include an evaluation milestone.

In the past century the number of medications administered to pregnant woman and to children has multiplied enormously. It is possible that at present the fetus is potentially at greater risk from the well-intentioned use of pharmaceuticals than from the stress of labor and delivery. Staff of OPPE, in collaboration with staff of Extramural Programs, is continuing to cooperate in devising a developmental pharmacology research program that is concerned with effects of medications administered during pregnancy and childbirth.

The facilities and resources activity of the OPPE has included participation in the development of plans for an intramural clinical research unit in reproductive research and perinatal biology to be activated in the Clinical Center, NIH. This program will be housed in an addition of 15,492 net square feet, made available through construction of the 8th, 9th, and 10th floors in wing G. Target date for occupancy is October, 1974. This activity has also been included in planning for remodeling of the existing Clinical Center Out Patient Department and for a larger future ambulatory care facility, as well as the design of a small temporary laboratory and animal facility near building 14G and minor rearrangement of 13N in the Clinical Center to adapt it to new program needs.

Interest of OPPE staff continues in research in child development and the man-made environment. A research conference was held in this area of concern in October, 1973, and work on the report is progressing. Staff of OPPE continue to exploit opportunities to explore new concepts in this area with a view to defining impact on the direction and progress of Institute programs.

During Fiscal Year 1974, the Institute designated a member of the OPPE staff to serve as the NICHD Evaluation Officer. This Evaluation Officer, in conjunction with Institute program staff, developed a NICHD Evaluation Plan for FY 1974-79. The plan emphasizes and seeks to strengthen the Institute's on-going evaluation activities and includes the development of outside consultant evaluation projects to be funded with DHEW set-aside evaluation funds.

Staff of OPPE continues to participate in coordination efforts, and to maintain liaison with agencies whose missions are complementary to or supportive of the Institute's objectives. During the current year these included the Interagency Panel on Early Childhood Research and Development; the Interagency Panel for Research and Development on Adolescence; the Interagency Task Force on Comprehensive Services for School-Age Parents (membership on the Steering Committee); the DHEW Interagency Committee on Planning for Children; the DHEW Interdepartmental Committee for Planning and Implementation of PL 91-695, Lead-Based Paint Poisoning Prevention Act, and its Subcommittee on Research and Statistics Issues (Chairman), and the Interagency Panel on Environmental Mutagenesis. An OPPE staff member also served as NICHD representative at the Organization Committee meetings for the Fogarty Center Conferences of Preventive Medicine, as Chairman of the NICHD Safety Committee, as Chairman of the NICHD Scientific Publications Steering Committee, and as NICHD liaison representative to the Council on Child Health of the American Academy of Pediatrics and to its Committee on Accident Prevention. Another continued activity this year is service of an OPPE staff person as a member of the Technical Advisory Committee, formerly advisory to the Food and Drug Administration, now transferred to the Consumer Product Commission. The Acting Associate Director, OPPE continues to represent the NICHD on the NIH Advisory Committee for Program Planning and Evaluation.

There are some new activities. The Acting Associate Director for OPPE, NICHD, has been named NIH representative on the DHEW Committee on Children recently created by the Secretary, HEW. Also an OPPE staff member was appointed to serve as a member of the FDA Ad Hoc Facilities Review Panel. Another OPPE staff member has been named to the new Intradepartmental Committee on Child Abuse and Child Neglect. He has been given the additional responsibility of planning and conducting a conference on Biomedical and Behavioral Research on Child Abuse.



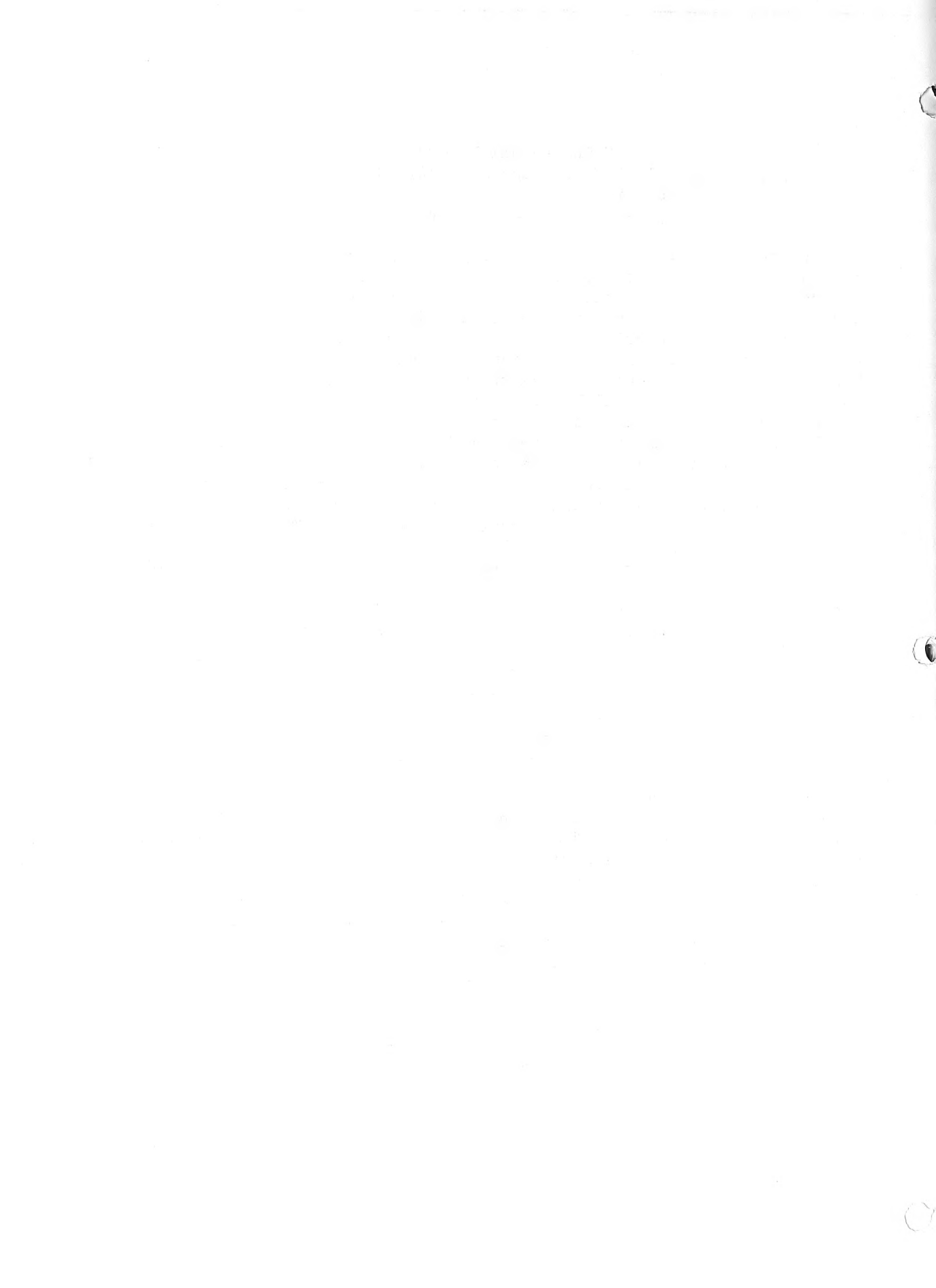
NICHD Annual Report
July 1, 1973 through June 30, 1974
Office of the Associate Director for
Epidemiology and Biometry

In the past year, the biometry and epidemiology areas, under the administrative direction and guidance of this office, have continued their activities of research and consultation. The growth of these activities has been somewhat hampered by our inability to obtain additional positions, particularly in the intramural consultation sphere where the Biometry Branch could use additional mathematical statisticians and scientific computer programmers. The Biometry Branch currently has two mathematical statisticians and no computer programmers available for statistical consultation with intramural scientists. One of these mathematical statisticians devotes much of his time to the Gerontology Research Center. In the past year, the frequency of requests by intramural scientists on the NIH campus for statistical consultation and service has jumped considerably. The Branch is embarrassed by being unable to complete the consultation in a thorough manner by also taking over the necessary calculations and computations of data. The statisticians have no computer programmer to work with in doing data analysis. In addition, with regard to the consultations themselves, the Branch Chief has had to increase the number of his own consultations with intramural scientists so that no one requesting services would be turned away. This is now building up to a bad situation unless remedied in the near future.

The Biometry Branch has had an excellent year. The staff in this Branch have published 14 articles in reputable scientific journals. Most of these have been reports of research in statistical methodology and theory and some have been applications of statistical techniques, particularly sophisticated time series procedures in a variety of substantive areas.

In addition, the Biometry Branch continues its highly important biostatistical studies and activities. The Branch continues to determine and assess the nature of adverse effects of oral contraceptive usage. It is in its full second year of serving as a statistical center for the collaborative study of the clinical trial on amniocentesis. The purpose of this trial is to determine adverse effects of the technique on both mother and baby up to one year of age. The Branch has also been significantly involved in the planning and design of another collaborative clinical trial being initiated by the Scientific Director of the Institute. This is to evaluate the side effects of phototherapy in the treatment of hyperbilirubinemia.

It will be noted in the Biometry Branch report that the Branch has been requested to give advice on statistical issues regarding design or analysis of experiments in a number of significant intramural scientific studies. In addition, members of the Branch serve as Project Officers on a number of contracts.

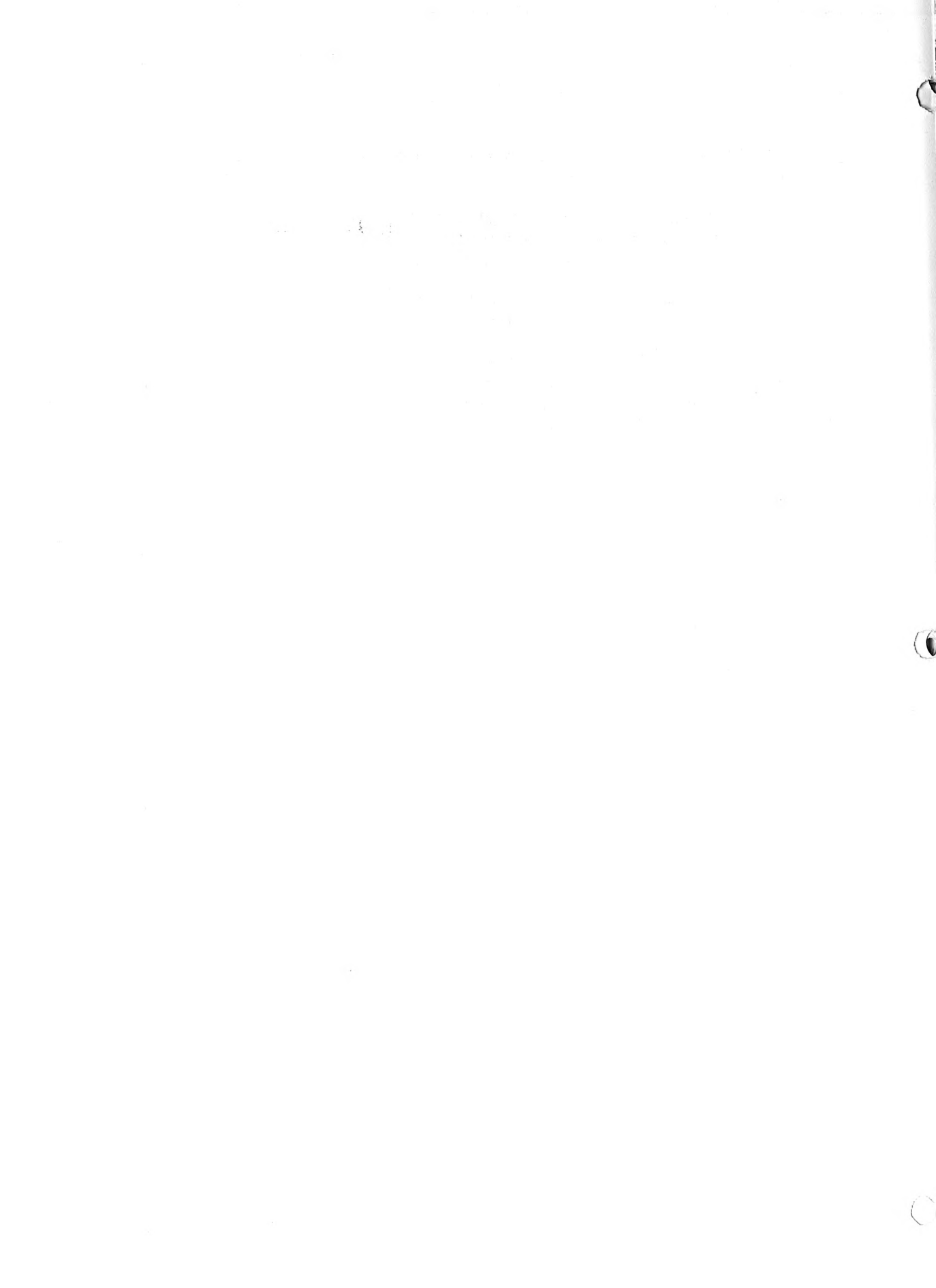


An invited lecture to the Epidemiology Department,
School of Public Health, Johns Hopkins University.

An invited lecture to the Statistics Department,
School of Business, New York University and to the
New York Statistical Association.

A formal discussant of the Presidential invited
address at the annual meeting of The Biometric
Society (ENAR).

In addition, the Associate Director was honored by being appointed a Johns
Hopkins University Centennial Scholar.



NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Epidemiology Branch

The Branch performs epidemiologic studies in areas related to the mission of the Institute and provides epidemiologic consultation to scientists in both the intramural and extramural programs of the Institute. At the present time Branch research activities focus on two segments of the Institute's interest: 1) the etiology and prevention of prematurity, and 2) the etiology and prevention of infant mortality. Some Branch projects aim at elucidating the factors which determine birth weight and length of gestation. Other projects are attempts to describe infant mortality trends and identify factors affecting the trends. Still other projects are concerned with the etiology of specific causes of infant mortality: 1) Respiratory Distress Syndrome, the most important single cause of death under 30 days of age, and 2) Sudden Infant Death Syndrome, the most important cause of postneonatal mortality.

Mr. Nathaniel B. White, Jr., temporarily left the Branch in September 1973, to commence training for a Ph.D. degree in biostatistics at the George Washington University in Washington, D.C. In July, 1973, Mrs. Inez Roberts, a STRIDE Trainee in biostatistics joined the Branch to assist Mr. White in four tasks related to his project concerned with tests for spread.

In November, 1973, Dr. Jacek J. Pietrzyk from Krakow, Poland, joined the Branch as a Guest Worker. During his six month stay in the Branch, Dr. Pietrzyk's accomplishments, which are documented elsewhere in this report, were numerous and varied.

Non-Project Activities:

During FY 74 a considerable amount of staff time was spent in "non-project" activities.

Branch Chief:

1. Served on the NICHD Scientific Publications Committee.
2. Continued his teaching activities in the Department of Pediatrics at Georgetown University Hospital.
3. Provided extensive consultation to the Perinatal Biology and Infant Mortality Branch (PBIM) staff in the development of contract proposals to study 1) the epidemiology of Sudden Infant Death Syndrome (SIDS), 2) developmental sleep physiology in infants of high and low risk for SIDS, and 3) autonomic regulation during sleep: developmental studies.
4. Provided consultation to staff of the Growth and Development Branch in the administration and execution of a contract to study the interactions of nutrition, infection and growth rate in the children of a Guatemalan village.

5. Traveled to Guatemala as a consultant to a research group under contract with the Growth and Development Branch.

Dr. Lundin:

1. Continued his staff appointment as Lecturer in the Department of Epidemiology at the Johns Hopkins School of Hygiene and Public Health.
2. Acted as consultant to the University of Louisville study of uterine cancer and cervical cytologic screening.
3. Served as a member of Scientific Committee Number 45 of the National Council on Radiation Protection and Measurements. This committee is concerned with occupational exposure to ionizing radiation.

Dr. Pietrzyk:

1. Studied all of "Epidemiology - Principles and Methods" by MacMahon and Pugh.
2. Wrote one complete manuscript entitled: "A Study of the HL-A System in Children with Congenital Malformations." This paper has been submitted to the Journal of Pediatrics.
3. Wrote two complete PL480 proposals for research projects to be undertaken upon his return to Poland:
 - a. "Major histocompatibility complex in children with increased disease susceptibility."
 - b. "HL-A system in children with CNS malformations."
4. Co-authored a manuscript with Dr. Spiers on the relationship between potato consumption and the occurrence of spina bifida and anencephaly.
5. Participated with Drs. Spiers and Stark in a study of secular mortality trends from solid tumors in the United States.
6. Gave a talk at Georgetown University. (See "Talks")
7. Completed the beginning NIH course in Fortran programming.

Dr. Spiers:

1. Served as Assistant Project Officer on the Gould and Wior contracts (see under Contract and Collaborative Research section).
2. Prepared the following manuscripts and scientific communications:
 - a. Letter to the Editor. "Multiple Ovulation and the Risk of Anencephaly and Spina Bifida." Lancet, ii:1149, 1973.

- b. "Water Hardness and Infant Mortality Rates in the United States." Accepted for publication in Pediatrics.
- c. "Estimated Rates of Concordancy for the Sudden Infant Death Syndrome in Twins." Accepted for publication in the American Journal of Epidemiology.

Mr. Ernest Harley:

1. Processed Brewer study data.
2. Created program to format Brewer data for CCSS System.
3. Linked two-number file with existing Brewer study file which enabled the information from county birth-death to be recorded.
4. Linked birth-death records to Brewer file. Reformatted birth-death records into Brewer-type records.
5. Created two programs for tabling of gestation periods and birth weights as functions of other parameters from Brewer study data.
6. Implemented CCSS System for batch processing on IBM 370.
7. Gave seminar on CCSS System (see "Talks").
8. Processed Perinatal data file to select multi-births and abnormal births.
9. Prepared Perinatal data to be used on CCSS System.
10. Wrote programs for Budget Officer to analyze data as to money spent in particular program areas and various CAN numbers.
11. Wrote program to compute statistics for annual LEO Statistical Report.

Mr. Samuel Jones:

1. Processed Brewer data.
2. Sorted data for various runs of the Brewer data tabling programs.
3. Helped implement CCSS on PDP-10.

Mrs. Inez Roberts:

1. Prepared computer programs and plotted graphs for book.
2. Computed t statistics for Dr. Hetzel's RFSH Study.

Mr. William Bell:

1. Processed Brewer study data.
2. Programmed edit routine to establish correct number of records for **Brewer** study.
3. Created several summary records for tabling programs.
4. Implemented CCSS System on PDP-10.
5. Structured Perinatal data for input CCSS System.

Mr. Terrance Corbin:

1. Processed Brewer study data.
2. Created reduced variable file from original perinatal file.
3. Formated Perinatal data to fit CCSS System.

Bibliography:

Archer, V.E., Wagoner, J.K. and Lundin, F.E., Jr.: Lung cancer among uranium miners in the United States. Health Physics 25:351-371, Oct., 1973.

Hoffman, H.J., Stark, C.R., Lundin, F.E., Jr., Ashbrook, J.D.: Analysis of birth weight, gestational age and fetal viability, U.S. births, 1968. Obstetrical and Gynecological Survey, accepted for publication as a supplement.

Stark, C.R., and Froggatt, P.: Epidemiologic Research. No. 5 in a series entitled: "Research Planning Workshops on the Sudden Infant Death Syndrome. DHEW Publication No. (NIH) 74-581, 1974.

Spiers, P.S. Letter to the Editor. "Multiple Ovulation and the Risk of Anencephaly and Spina Bifida." Lancet, ii:1149, 1973.

Spiers, P.S., Wright, S.G., and Seigel, D. "Water Hardness and Infant Mortality Rates in the United States." Pediatrics (accepted for publication).

Spiers, P.S. "Estimated Rates of Concordancy for the Sudden Infant Death Syndrome in Twins." American Journal of Epidemiology (accepted for publication).

Talks:

Stark, C.R.: Epidemiologic Considerations in Prospective Studies. NICHD-NHLI sponsored meeting entitled, "Possible Pharmacologic Mechanisms for Enhancement of Lung Maturation for Prevention or Treatment of Neonatal Respiratory Distress Syndrome. Belmong, Elkridge, Maryland April 2, 1974.

Pietrzyk, J.J.: III-A Systems in Children with Congenital Malformations. Department of Pediatrics Seminars in Immunology Georgetown University, Washington, D.C., January 10, 1974.

Harley, E.E.: Conversational Computer Statistical System, An Approach for Processing and Analyzing Statistical Data. Biometrics Seminar Series, National Institutes of Health, Bethesda, Maryland April 24, 1974.

Serial No. _____
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Low-Weight-for-Gestational-Age in the U.S.

Previous Serial Number: HD-EB-3

Principal Investigator: Frank E. Lundin, Jr., M.D.

Other Investigators: Howard J. Hoffman, M.A., Charles R. Stark, M.D.
J. Douglas Ashbrook, M.A.

Cooperating Units: National Center for Health Statistics

Man Years: Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives:

The data for this study make possible the simultaneous classification of births by birth weight and gestational age calculated from the date of last menstrual period as collected by 36 states and the District of Columbia. These characteristics of the data permit us to establish norms for birth weight by gestational-age for a general population and to describe the risk of low-weight-for-gestational-age babies by race, sex, maternal age, parity, month of birth and geographic area. For 1968 but not for 1969 and 1970, single and plural live births can be studied separately. Starting in 1969, maternal education is also available so as to permit study according to this measure of social status. The risk of fetal wastage in relation to low-weight-for-gestational-age can be studied by the above mentioned characteristics.

Progress:

Extensive tabulations of single live births and fetal deaths have been prepared by the Division of Computer Research and Technology, (DCRT). Sophisticated analytic techniques have been programmed in collaboration with DCRT and have been used for the analysis of substantial portions of the data. An extensive manuscript has been accepted for publication. Additional publications are planned from existing tabulations and from analyses in progress.

Significance to Biomedical Research and Program of the Institute:

Clinically low-weight-for-gestational-age babies have increased morbidity and statistically they have much increased mortality. This descriptive study provides epidemiologic leads to factors responsible for infant mortality. Related studies planned by the Branch will allow direct estimates of mortality in low-birth-weight-for-gestational-age babies.

Proposed Course:

Data for live births in 1970 and fetal deaths in 1969 and 1970 are being acquired. Tabulation and analysis of twin data for 1968 are in progress. Tabulations for the 1969 and 1970 data are planned during the remainder of this FY. Further analyses and manuscript preparation will continue next FY.

Honors and Awards: none

Publications: Hoffman, H.J., Stark, C.R., Lundin, F.E., Jr., and Ashbrook, J.D.: Analysis of birth weight, gestational age and fetal viability, U.S. births, 1968. Obstetrical and Gynecological Survey, accepted for publication as a supplement.

Serial No. _____
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Relative Efficacy of Two Prenatal Care Regimens

Previous Serial Number: HD-EB-4

Principal Investigator: Frank E. Lundin, Jr., M.D.

Other Investigators: Charles R. Stark, M.D., Charles U. Lowe, M.D.

Cooperating Units: Contra Costa County Medical Services
State of California - Human Relations Agency
Alameda - Contra Costa Medical Association

Man Years:

Total: 1.6 years
Professional: 1.6 years
Other: none

Project Description:

Objective:

To determine whether pregnant medically indigent women indoctrinated by nutritional lectures and by the physician at each prenatal visit bear healthier babies and have less morbidity than comparable women treated with a traditional regimen of weight restriction, diuretics and low calorie-low salt diets. The women treated with nutritional education were not subjected to weight control, diuretics nor low calorie-low salt diets.

Methods Employed:

Initially (Phase I) the treatment group was compared with medically indigent deliveries in the same hospital. A data procurement contract was used to obtain coded abstracts for 5,615 case records of prenatal care and deliveries at Contra Costa County Hospital over a period of 5-1/2 years. Records of infant deaths among these births were obtained from the State of California, Department of Public Health. Later additional control groups were obtained to answer several important questions with regard to possible effects of the treatment (Phase II). Birth weights and gestational ages before and in the early part of the treatment period were studied by abstraction and tabulation of records for a 25-percent sample of births at Contra Costa County Hospital for the 4-1/2 year period prior to Phase I. To evaluate and correct for bias due to differential useage of Medi-Cal payment to private hospitals, computer records of births to residents of

Alameda and Contra Costa Counties were obtained from the State of California. These records contain birth weight and infant mortality and identify deliveries financed by Medi-Cal during the years 1967, 1968, and 1970. Finally data for a random sample of approximately 600 Medi-Cal deliveries in Contra Costa County were collected from private hospitals and private physicians under the data procurement contract. Information which was coded is comparable to that for Contra Costa County Hospital deliveries in Phase I. End points include prenatal morbidity, infant and maternal hospital morbidity as well as birth weight and gestational age. Code sheets for Phases I and II were forwarded to NICHD for processing and analysis to achieve the objectives of the study.

Progress:

Data for Phase I have been edited, corrected and substantially analyzed. Birth weight and mortality data for Phase II have been analyzed. Data for the Medi-Cal sample of approximately 600 race-matched deliveries in private hospitals has been abstracted but not processed. Two manuscripts which describe completed analyses are in preparation. One details the effects of the treatment on birth weight, gestational age and fetal and infant mortality; while the other describes the effects on maternal and infant morbidity.

Significance to Biomedical Research and Program of the Institute:

It is anticipated that this study will show that avoidance of gestational weight control, avoidance of drugs, and special attention to nutrition will result in no adverse effects to the infant and absence of the "toxemia of pregnancy" syndrome.

Proposed Course:

Two manuscripts will be submitted for publication this FY. Additional analyses will be done as time permits.

Honors and Awards: none

Publications: none

Serial No. HD-EB-10

1. Epidemiology Branch

2. ---

3. Bethesda, Maryland

PHS-NIH
Individual Project Reports
July 1, 1973 through June 30, 1974

Project Title: Multiple Ovulation and Risk of Anencephaly and Spina Bifida

Previous Serial Number: HD-EB-10

Principal Investigator: Philip S. Spiers, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years:

Total: 10 weeks

Professional: 10 weeks

Other: None

Project Description:

Objectives:

To examine the epidemiology of Anencephaly/Spina Bifida (ASB) and dizygous twinning rates.

Summary:

Contrary to the report by Stevenson et al. to the effect that dizygous twinning rates and ASB are positively associated, the present investigation tends to find them negatively associated. It is suggested that one risk factor for the occurrence of ASB is a subnormal level of gonadotropin.

Significance to Biomedical Research and Program of the Institute:

The role of hormones in the production of congenital malformations should be explored.

Honors and Awards: None

Publications: Lancet, 1973, (ii), 1149.

Serial No. HD-EB-11

1. Epidemiology Branch

2. ---

3. Bethesda, Maryland

PHS-NIH
Individual Project Reports
July 1, 1973 through June 30, 1974

Project Title: Infant Mortality Rates and Hardness of Local Water Supplies

Previous Serial Number: HD-EB-11

Principal Investigator: Philip S. Spiers, Ph.D.

Other Investigators: Daniel Seigel, S.D., Stephen G. Wright,

Cooperating Units: Biometry Branch, NICHD

Man Years:

Total: 9 weeks

Professional: 9 weeks

Other: None

Project Description:

Objective:

To seek confirmation of British study which reported a negative correlation between hardness of water and infant mortality rates.

Summary:

Using complete data from as many of the 100 largest U.S. cities no significant correlation could be found between infant mortality rates and hardness of water. The lack of significant findings persisted when other factors such as income level, rainfall, etc., were controlled for.

Significance to Biomedical Research and Program of the Institute:

The British results are probably due to a secondary association.

Honors and Awards: None

Publications: Pediatrics (accepted for publication)

Serial No. HD-EB-12

1. Epidemiology Branch

2. ---

3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Estimated Concordancy Rates for the Sudden Infant Death Syndrome among Twins (Formerly entitled "The Susceptibility of Twins to the Sudden Infant Death Syndrome")

Previous Serial Number: HD-EB-12

Principal Investigator: Philip S. Spiers, Ph.D.

Other Investigators: Karen Fetterly

Cooperating Units: Biometry Branch, NICHD

Man Years:

Total: 8 weeks

Professional: 8 weeks

Other: None

Project Description:

Objective:

To examine the hypothesis suggested from review of the literature that the concordancy rates are the same for like and unlike twins.

Summary:

Using infant death certificates and matched birth certificates together with certificates for all births occurring in North Carolina 1959-67, it was found that the concordancy rates do not differ.

Significance to Biomedical Research and Program of the Institute:

Results strongly suggest that the foetal genotype plays no significant role in the etiology of this condition.

Honors and Awards: None

Publications: American Journal of Epidemiology (accepted for publication)

Serial No. HD-EB-14
1. Epidemiology Branch
2. ---
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Sudden Infant Death Syndrome in the United States:
a study of geographic and other variables.

Previous Serial Number: None

Principal Investigator: Philip S. Spiers, Ph.D.

Other Investigator: James J. Schlesselman, Stephen G. Wright

Cooperating Units: Biometry Branch, NICHD

Man Years:

Total: 30 weeks
Professional: 30 weeks
Other: None

Project Description:

Objectives:

To examine evidence for any geographic variation of the sudden infant death syndrome within the United States.

Summary:

It was estimated that in 1958 the rate of SIDS in the United States was 2.2 per 1000; by extrapolation it was estimated that by 1969 this had fallen to 1.5 per 1000 live births. The number of estimated deaths were 9,278 and 5,515 respectively. Among white infants, SIDS death rates were positively correlated with longitude. This association was not observed among black infants.

Significance to Biomedical Research and Program of the Institute:

SIDS death rates are probably falling - most probably due to reduced exposure to infectious agents. The association that white SIDS rates have with longitude is opposite to that found for spina bifida. The question arises as to whether this is a mere coincidence.

Honors and awards: None

Publications: Submitted

Serial No. HD-EB-15
1. Epidemiology Branch
2. ---
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Secular Trends in Twinning Rates and Infant Death Rates
from Congenital Malformations

Previous Serial Number: None

Principal Investigator: Philip S. Spiers, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years:

Total: 6 weeks
Professional: 6 weeks
Other: None

Project Description:

Objectives:

To examine the prediction that although the rates for both conditions are declining, the malformation death rate will fall most precipitously in those states exhibiting the least decline in twinning rates.

Summary:

Prediction is confirmed.

Significance to Biomedical Research and Program of the Institute:

Further support to hypothesis that pituitary hormone level is a contributory factor in the etiology of neural tube defects.

Honors and Awards: None

Publications: None

Serial No. HD-EB-16
1. Epidemiology Branch
2. ---
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Potato Consumption and Neural Tube Malformations

Previous Serial Number: None

Principal Investigator: Philip S. Spiers, Ph.D.

Other Investigator: Jacek Pietrzyk, Joyce Piper and Donna Glebatis

Cooperating Units: New York State Department of Health, Birth Defects
Institute

Man Years:

Total: 20 weeks
Professional: 20 weeks
Other: None

Project Description:

Objectives:

To examine by means of potato consumption habits Renwick's hypothesis that neural tube defects are carried by the ingestion during pregnancy of blighted potatoes.

Summary:

By means of introductory correspondence and telephone interviews a survey was made of the current potato consumption habits of 265 mothers of infants born with neural tube defect over the period January, 1970 - December, 1972, and 549 matched control mothers. Both cases and controls were obtained from birth certificate entries. Renwick's hypothesis was tested by subjecting responses to seven questions to a modified χ^2 test. The results did not support the hypothesis. In 6 out of 7 instances the direction of the responses was opposite to that predicted under the hypothesis.

Significance to Biomedical Research and Program of the Institute:

Results are consistent with those of other investigators who have attempted to confirm the hypothesis. Prospective tests of Renwick's hypothesis upon human populations do not at this stage seem justified.

Honors and Awards: None

Publications: Submitted

Serial No. HD-EB-17

1. Epidemiology Branch
2. ---
3. Bethesda, Maryland

PHS-NIH
Individual Project Reports
July 1, 1973 through June 30, 1974

Project Title: Permutation Test for Spreads

Previous Serial Number: None

Principal Investigator: Nathaniel B. White, Jr.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 8 weeks
Professional: 3 weeks
Other: 5 weeks

Project Description:

Objectives:

We will investigate a permutation test for spreads, find the power and investigate its sensitivity to translations, asymptotic properties, and make comparisons with other tests for spread.

Summary: The project is just beginning.

Significance to Biomedical Research and Program of the Institute:

Results could be used in the analysis of biomedical data to test for spread, in situations where the assumption of normality is invalid.

Honors and Awards: None

Publications: None

Serial No. HD-EB-18
1. Epidemiology Branch
2. ---
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Modified Rosenbaum's One and Two Layers Test

Previous Serial Number: None

Principal Investigator: Nathaniel B. White, Jr.

Other Investigator: None

Cooperating Units: None

Man Years:

Total: 20 weeks
Professional: 8 weeks
Other: 12 weeks

Project Description:

Objectives:

To calculate the exact probabilities for Kamat's modification on Rosenbaum's one-and two-level test, a Mann - Whitney like test for differences in spread. The power and sensitivity to translation of the tests will be studied.

Summary:

The programs to calculate the probabilities are being formulated and debugged. The power and the sensitivity to translation will be explored after the programs are completed.

Significance to Biomedical Research and Program of the Institute:

Results could be used in the analysis of biomedical data to test for spread, in situations where the assumption of normality is invalid.

Honors and Awards: None

Publications: None

Serial No. HD-EB-19
1. Epidemiology Branch
2. ---
3. Bethesda, Maryland

PHS-NIH
Individual Project Reports
July 1, 1973 through June 30, 1974

Project Title: Monte Carlo Procedures for Modified Rosenbaum Tests

Previous Serial Number: None

Principal Investigator: Nathaniel B. White, Jr.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 10 weeks
Professional: 2 weeks
Other: 8 weeks

Project Description:

Objectives:

To obtain the probabilities for the three-, four-, five-, etc., level modified Rosenbaum's tests employing Monte Carlo procedures.

Summary:

The programs are being formulated and debugged.

Significance to Biomedical Research and Program of the Institute:

Results could be used in the analysis of biomedical data to test for spread, in situations where the assumption of normality is invalid.

Honors and Awards: None

Publications: None

NICHD Annual Report
July 1, 1973 through June 30, 1974

Epidemiology Branch
Contract and Collaborative Research

Contract Title: Relationship of Heterozygosity in Phenylketonuria to Intelligence Quotient

Contractor: University of Southern California in Los Angeles, California

Money Allocated: \$30,830.00

Objectives: To determine whether heterozygosity to phenylketonuria is associated with a decrease in intelligence.

Summary: Carrier status to phenylketonuria among siblings of phenylketonurics will be determined by phenylalanine loading test. The intelligence scores obtained by standard intelligence tests will be compared for PKU heterozygotes and their genotypically normal siblings. This evaluation is planned to include about 100 siblings. It is hypothesized that the heterozygous fetus in utero, because of his partial deficiency in tyrosine synthesis, may be susceptible to damage from maternal dietary protein deficiency. In this situation neither the mother nor the fetus is able to compensate for deficiencies in maternal tyrosine intake by hydroxylation of phenylalanine to tyrosine.

Significance to Biomedical Research and Program of the Institute: The investigation relates to the Institute's vigorous efforts to pursue testable hypotheses in the area of "non-specific" mental retardation and compliments other studies on nutritional and metabolic factors in mental retardation currently being explored by the Epidemiology Branch.

Project Officer: Dr. Heinz Berendes

NICHD Annual Report
July 1, 1973 through June 30, 1974

Epidemiology Branch
Contract and Collaborative Research

Contract Title: The Epidemiology of Birth Weight with Particular Reference to the Effect of Induced Abortion on Subsequent Offspring

Contractor: Mother and Child Institute, Warsaw, Poland
Money Allocated: 1,450,785 Zlotys (first year)

Objectives: To determine whether or not therapeutic abortion affects the birthweights of subsequent offspring.

Summary: Controlling for a number of factors already known to be related to low birth weight, this study will assess the relationship between previous abortion and the occurrence of low birth weight in subsequent offspring. Information will be gathered on all pregnant women attending prenatal clinics, both during the actual pregnancy and during the first 3 months of the infant's life. Some 18,000 women will form the basis for the study.

Significance to Biomedical Research and Program of the Institute: Since birth weight is such an overriding determining risk factor for infant mortality, and since therapeutic abortion is the predominant method of birth control in many countries the results may affect future policies.

Proposed Course: During the first 9 months a pilot phase of the study will be performed. The design should be compatible with that already in use by the WHO which is examining the same question in Yugoslavia and Singapore. Continuation of this project will be subject to the satisfactory completion of the pilot phase.

Unfortunately progress on this study was impeded by the temporary withholding of PL 480 funds for Polish projects. In order that the study might nevertheless continue on the time schedule that was conducive to the aims of the wider study being conducted by WHO, the latter agreed to finance the project for the first six months and perhaps the first year.

Project Officer: Charles R. Stark, M.D.

Assistant Project Officer: Philip S. Spiers, Ph.D.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Biometry Branch

The personnel of the Biometry Branch have been primarily engaged in three activity areas. The first is to perform the functions of a data center for two national collaborative studies of obstetric and neonatal practice, amniocentesis and phototherapy for bilirubinemia.

The second activity is statistical consultation to professional personnel in the Institute. Demands for such consultations are now in excess of our resources. We have neither a sufficient number of statisticians nor of data processing personnel to serve this need.

Finally, the staff continues to conduct research activities of its own, most of it consisting of new statistical methodology devised for consultations performed within the Institute.

Specific Activities of the Branch

A. Data Center Functions

The Branch serves the role of a data center for a national multicenter study of the risks associated with amniocentesis, a surgical procedure used during pregnancy. Forms were being received at maximum volume this last year. Staff was occupied with preparing high quality tapes, and with generating tables of some of the immediate complications. In the coming year the study should continue to demand considerable staff effort, with a greater percentage of time given over to analysis of the data.

Responsibility for the statistical aspects of a clinical trial of phototherapy as a treatment of bilirubinemia in the newborn was also assumed by the Branch during this year. A randomization scheme was devised, and plans were made for processing of forms that will start to be received in mid 1974.

B. Research

The research interests of Branch members are reflected by their publications and by the contracts initiated from within the Branch. Out of our consultations with the Gerontology Research Center, statistical techniques were developed for planning of longitudinal studies. Because we are frequently called upon to design studies of the side effects associated with agents such as the oral contraceptive, and because the side effects of interest are often rare, the Branch has been active in research on sample size determinations, design, and methods of analysis appropriate for case-control studies.

Collaboration with members of the Epidemiology Branch has led to publications on congenital malformations, sudden infant death syndrome, and birth weight.

Finally, one member of the Branch has been especially interested in the application of time series theory to biomedical problems.

The contracts in which members of the Branch serve as project officers are extensions of our research activities. A series of contracts on the adverse effects of use of the oral contraceptive are now nearing completion. Publications emanating from them will indicate that breast cancer cases now being diagnosed do not appear to be associated with use of these agents, but that stroke does appear to occur more frequently among users of oral contraceptive users, just as do other thromboembolic disorders.

The Branch has also initiated a contract to monitor the incidence of a wide variety of diagnoses in the newborn. The source of the data is discharge diagnoses on approximately one million births submitted to the Commission on Professional Hospital Activities, in Michigan (CPHA). CPHA will submit tabulations on a monthly basis to the Center for Disease Control, in Atlanta, where a system to detect "epidemics," particularly of congenital malformations, is being developed. The Branch had an important role in developing this system, and sees it as an experiment on whether epidemics can be promptly detected, and whether tragedies such as that caused by the drug thalidomide can be reduced in scale. Further, we view it as one way of sensing the impact of a wide range of new environmental pollutants to which we are becoming increasingly exposed.

A study of the side effects of abortion comparing curettage with suction was completed this year and led to a monograph reporting its results. One member of the Branch was particularly involved in studies of the role of repeated abortions on the risk of ectopic pregnancy, a substudy of this project.

C. Consultations

One of the important functions of the Branch is to provide consultative support to staff of the Institute requiring it. It is apparent that our small staff is now better known throughout the Institute since requests for their services are now rather frequent.

Most consultations come from Intramural scientists and require our assistance over short intervals on very limited problems. Sometimes, though, they involve larger data sets, and may extend over longer periods of time.

Extramural scientists also call on us rather frequently, primarily with respect to the review and monitoring of contracts. Our statisticians are very active in the programs of aging, perinatal biology, The Center for Population Research, and the Epidemiology Branch.

A list of some of the more significant consultations follows:

1. Provided sample size analysis, randomization scheme and assisted in the planning and logic of a randomized, double blind, controlled clinical trial of a newly developed vaccine for H. influenzae type b meningitis.
John B. Robbins

2. Provided experimental design and sample size analysis for a proposed study of effects of estrogen administration to post-menopausal women on the serum protein Te_{8g}. Design permits estimation of the treatment effects of various doses of estrogen, their residual or carry-over effects, if any, the effects of the order of dose administration and the patient responses. Mortimer B. Lipsett

3. Provided analysis to determine the number of subjects and the number of replications per subject for a study of the effects of varying Ph, osmolality and centrifugation time on cell counts in urine. John Rowe

4. Analysis of U.S. 1968 single live births and fetal deaths. Many conventional statistical summary techniques by birth weight and gestational age categories have been applied to these data. In addition, contour analysis of each sub-population as classified by sex, metropolitan status and race, has also been applied, yielding several new insights into the birth weight-gestational age continuum. C. R. Stark, F. E. Lundin, Jr., and J. D. Ashbro

5. Life table construction on barrier-reared Fischer's 344 rats. Age-specific pathology rates and life table characteristics have been derived for this colony of aging rats maintained at Charles River Animal Laboratories Wilmington, Massachusetts. D. C. Gibson and Albert Jonas

6. Gonadotrophin measurements in adolescent and pre-adolescent humans. Urine measurements of LH and FSH have been collected for sixty consecutive days during a replicated, designed experiment. Analysis of covariance, smooth trend analysis and various statistical procedures are being performed on these data. J. W. Hansen and G. T. Ross.

7. Investigation of seasonality and/or periodic variation in the potency of pertussis vaccine standards. These time series derive from bio-assay standards maintained by the Bureau of Biologics for more than 15 years in an effort to monitor industry production of the vaccine. Approximately 40% of the regular weekly values are "missing." The analysis of time series data with such a high rate of missing data requires specially tailored algorithms, and modified confidence band procedures. C. R. Manclark and C. J. Maloney

8. The significance of weight loss in the evaluation of pituitary response to LH-RH in women with secondary amenorrhea. Linear and polynomial regression analyses were performed to relate response measures to percent of ideal body weight longitudinally for women in the study. R. L. Van de Wiele

NICHD Annual Report
July 1, 1973 through June 30, 1974
Biometry Branch

Contracts

Members of the Branch serve as primary project officer on five contracts. The progress of the contracts is described below.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Biometry Branch

Contract and Collaborative Research

Contract Title : Epidemiologic Study of Breast Cancer and Benign Breast
Lesions in Relation to the Use of Ovarian Hormones
Contractor : Institute of Oncology, Ljubljana, Yugoslavia
Money Allocated : 524,460 Yugoslavian Dinars

Objectives: To determine whether women who use oral contraceptives have an increased risk of breast cancer.

Summary: This study is similar in design to contracts funded by our Institute in the United States. Women with benign and malignant breast tumours are interviewed concerning their previous use of steroid contraceptives. A matched group of patients without breast disease will also be interviewed as well as a group with benign breast disease that are not biopsied. Analyses will be done to determine whether any increased risk of breast disease exists in women who use steroid hormones.

Significance to Biomedical Research and Program of the Institute: This study is an important part of the Institute's program to monitor the side effects that have been proposed as potential sequelae to oral contraceptive use.

Proposed Course: Present plans are to terminate the study in 1975.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Biometry Branch

Contract and Collaborative Research

Contract Title : Birth Defects Monitoring Program
Contractor : The Center for Disease Control, Atlanta, Georgia
Money Allocated : \$298,560

Objectives: To determine whether a monitoring system can be developed to monitor and investigate outbreaks of congenital malformations and other disorders diagnosed in the newborn.

Summary: At the present time, The Commission on Professional Hospital Activities (CPHA) receives abstracts on all discharge diagnoses for newborns in hospitals in which approximately one third of U. S. births occur. For the purposes of this contract, tables will be provided to the Center for Disease Control (CDC) which will continually review them for unusually high rates of selected diagnoses. Where high rates occur, field investigations will take place to determine their causes.

Significance to Biomedical Research and Program of the Institute: This study is a natural extension of the interest of the Institute in the etiology of diseases of the newborn.

Proposed Course: The intent of the project officer is to continue this contract until the feasibility of this approach to monitoring epidemics of congenital malformations is adequately evaluated.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Biometry Branch

Contract and Collaborative Research

Contract Title : Oral Contraceptives and Tumors of the Breast
Contractor : University of California School of Public Health
Money Allocated : No money allocated in this fiscal year

Objectives: To determine whether the incidence of tumors of the breast is associated with oral contraceptive use.

Summary: The frequency of oral contraceptive use in women with breast cancer and women with benign breast lesions will be compared with that of control groups of women free of cancer and/or breast lesions. Cases diagnosed in a two-year period will be selected.

Three groups of women are being used:

- 1) Women with breast cancer 49 years of age or younger in whom cancer of the breast has been diagnosed for the first time during the study period. These are patients admitted consecutively to certain designated hospitals in the San Francisco Bay Area.
- 2) Women with benign breast lesions. These are women biopsied or operated on for benign lesions of the breast in the same hospitals within a specified period of the cancer cases. One woman with a benign breast lesion is matched with each cancer by age, by race, by religion, and by socio-economic status.
- 3) Women without evidence of breast lesions or cancer of any site. These women serve as controls. Two controls are chosen for each cancer case, one from surgical and one from the medical service.

The aim is to accumulate at least 400 cases in each of the study groups, women with breast cancer, with benign breast lesions, medical controls and surgical controls.

Basic data necessary for the study are derived from two main sources--review of the hospital record of each subject and a personal interview. The interview seeks information on socio-demographic, menstrual and reproductive and contraceptive variables.

Over 300 cases of malignant breast cancer have been studied, as well as appropriate controls. Preliminary tabulations have been made available to the Institute, so that current surveillance of this important problem can be maintained.

Significance to Biomedical Research and Program of the Institute: This study is an important part of the Institute's program to monitor the side effects that have been proposed as potential sequelae to oral contraceptive use.

Proposed Course: The study will expire in December 1974, with no further funding, at which time the results of the study will be published.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Biometry Branch

Contract and Collaborative Research

Contract Title : A Retrospective Study of the Risks for Cancer of the Breast, Body of the Uterus, Ovary, and Cervix among Users of Contraceptives
Contractor : Yale University, New Haven, Connecticut
Money Allocated : No money allocated this fiscal year

Objectives: To determine whether the incidence of carcinoma of selected sites is associated with oral contraceptive use.

Summary: Cases are being identified of women with breast, uterine, and ovarian cancer admitted to hospitals in the areas in and around Bridgeport, Hartford, and New Haven, Connecticut, and Springfield, Massachusetts, over a two-year period.

Controls are selected as the next patient with the appropriate matching variables admitted to the same hospital as the case with an acute medical or surgical condition or for an elective operation.

Interviews are administered in hospital, whenever possible, inquiring of demographic variables, obstetric history, menstrual history, contraceptive history, and a checklist of diseases.

Analysis will involve an examination of the difference in the proportions of cases and controls exposed to oral contraceptives for different lengths of time, for each site and for specific cell-types at each of the four sites. Relative risks for various lengths of exposure to the oral contraceptive will be calculated. Nearly 200 cases have been interviewed. Preliminary tabulations have been made available to the Institute, so that current surveillance of this important research problem can be maintained.

Significance to Biomedical Research and Program of the Institute: This study is an important part of the Institute's program to monitor the side effects that have been proposed a potential sequelae to oral contraceptive use.

Proposed Course: The study will expire in December 1974, with no further funding, at which time the results of the study will be published.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Biometry Branch

Contract and Collaborative Research

Contract Title : A Collaborative Study of Oral Contraception and
Cerebrovascular Disease
Contractor : Duke University Medical Center
Money Allocated : No money allocated in this fiscal year

Objectives: To determine whether the incidence of stroke in young women is increased by use of oral contraceptives, and if so to identify characteristics that are especially related.

Summary: The neurologic services of ten university medical centers have identified all young women admitted to their hospitals with definite or suspected cerebrovascular disease of any type. Four of the participating hospitals also identify patients admitted to each of the community hospitals in their metropolitan areas (Miami, St. Louis, Baltimore, and Atlanta). An estimated 200 patients with cerebrovascular disease are submitted for study each year. Clinical data on each of these patients are abstracted from the hospital and physician's records. The frequency of usage of oral contraception in these patients will be compared with that found in two selected control samples: 1) women hospitalized for other diseases and 2) women representing a "healthy" population residing in the same neighborhood as the patients selected for the study. Information regarding pill usage will be obtained from the patient and control subjects by personal interviews carried out in their homes.

All patients and controls have been selected and interviewed this year. All clinical data have been assembled at Johns Hopkins, where a coding system has been developed.

A manuscript was published in the New England Journal of Medicine in which the basic finding, a nine-fold increase in risk of stroke for oral contraceptive users, was published. Additional analyses on further risk factors in such women, are planned.

Significance to Biomedical Research and Program of the Institute: The study is an extension of previous research on thromboembolism and oral contraceptive use in which very few stroke cases were included and is a part of our program to monitor the significant effects on health of currently used contraceptives.

Proposed Course: The study will expire in December 1974, with no further funding, at which time the results of the study will be published.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Publications and Talks

Publications

1. Orr, W.C., Hoffman, H.J., and Hegge, F.W.: Sleep loss, sustained performance, and the basic rest-activity cycle (BRAC). In Chase, M.H., Stern, W.C., and Walter, P.L. (eds.): Sleep Research. Los Angeles, Calif., Brain Information Service, Brain Research Institute, U.C.L.A., Volume 2, 1973.
2. Orr, W.C. and Hoffman, H.J.: A 90-min cardiac biorhythm: Methodology and data analysis using modified periodograms and complex demodulation. IEEE Trans. on Biomedical Engineering, BME-21(2):130-143, 1974.
3. Hoffman, H.J., Guerry, IV, D., and Dale, D.C.: Analysis of cyclic neutropenia using digital band-pass filtering techniques. J. Interdisciplinary Cycle Research 5:1-18, 1974.
4. Ciaranello, R.D., Hoffman, H.J., Shire, J.G.M., and Axelrod, J.: Genetic regulation of the catecholamine biosynthetic enzymes II. Inheritance of tyrosine hydroxylase, dopamine- β -hydroxylase and phenylethanolamine N-methyltransferase. J. Biological Chemistry, 1974. In press.
5. Hoffman, H.J. and Ciaranello, R.D.: Genetic regulation of the catecholamine biosynthetic enzymes: Statistical appendix. J. Biological Chemistry, 1974. In press.
6. Orr, W.C., Hoffman, H.J., and Hegge, F.W.: Methods of biorhythmic analysis in the assessment of human performance. Annals of the New York Academy of Sciences, 1974. In press.
7. Hoffman, H.J., Stark, C.R., Lundin, Jr., F.E., and Ashbrook, J.D.: Analysis of birth weight, gestational age and fetal viability, U.S. births, 1968. Obstetrical and Gynecological Survey, (Supplement), 1974. In press.
8. Orr, W.C., Hoffman, H.J., and Hegge, F.W.: Ultradian rhythms in extended performance. J. Aerospace Medicine, 1974. In press.
9. Schlesselman, J.J.: Data transformation in two-way analysis of variance. J. Amer. Stat. Assoc. 68:369-378, 1973.
10. Schlesselman, J.J.: Planning a longitudinal study: I. Sample size determination. J. Chron. Dis. 26:553-560, 1973.
11. Schlesselman, J.J.: Planning a longitudinal study: II. Frequency of measurement and study duration. J. Chron. Dis. 26:561-570, 1973.

12. Schlesselman, J.J.: Sample size requirements in cohort and case-control studies of disease. Amer. J. Epid. In press.
13. Seigel, D.G. and Greenhouse, S.W.: Multiple relative risk functions in case-control studies. Amer. J. Epid. 97:324-331, 1973.
14. Seigel, D.G. and Greenhouse, S.W.: Validity in estimating relative risk in case-control studies. J. Chron. Dis. 26:219-225, 1973.

Talks

1. Schlesselman, J.J.: Multiple regression in the analysis of counts and proportions, The Washington Statistical Society, October 1973.
2. Hoffman, H.J.: Modern techniques of statistical time series analysis. Sponsored by the Washington Statistical Society, 16 May 1974, National Bureau of Standards, Gaithersburg, Maryland.
3. Hoffman, H.J.: Techniques of profile analysis, and uses of life table methods for animal models in aging research. Delivered at the "Conference on Animal Model Data Development for Aging Research, 3 June 1974, Jackson Laboratories, Bar Harbor, Maine.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Office of Associate Director for Program Services

SUMMARY

The Office of Program Services experienced a significant increase in workload factors over the previous fiscal year which affected the two branches constituting the Office: the Program Statistics and Analysis Branch and the Grants and Contracts Management Branch. The increase in workload was accompanied by a reduction of six full-time permanent positions to an approved level of 65 through June 30, 1974.

The Institute's extramural projects, including grant and contract programs increased over fiscal year 1973 by 385 projects and \$28.8 million. The increases were related to two events: (1) the release of fiscal year 1973 impounded funds (\$16.1 million) and the apportionment of fiscal year 1974 Congressional Increases (\$15.8 million). The release of fiscal year 1973 impounded funds cancelled out the phase-out of the old training programs and permitted them to continue close to their recommended level. At the same time the Institute inaugurated the new institutional fellowship program. The Research Career Award program was transferred from the Research Fellowships Budget Activity to a special line item under the Research Grants Budget Activity. It became necessary to fund for multi-years a number of research grants in order to lessen the impact of fiscal year 1974 competing funds on fiscal year 1975 President's Budget noncompeting funds. The workload related to the events above took place between February through June 1974, a relatively short period of time.

The Office was particularly pleased with the announcement that Pediatrics had accepted a publication entitled "Recipients of Research Grants from NICHD" which was prepared by staff of the Program Statistics and Analysis Branch. The Branch also was responsible for the publication of "The Extramural Program of Research on Aging in NICHD" and "Inventory of Federal Population Research." The Branch took the initiative to integrate the Division of Research Grants (DRG) machine record system with its own data system in order to avoid duplication of coding procedures and release time for analytical reporting. A study of the Branch by the Management Analysis Branch, ODA M, NIH, was conducted late in the fiscal year to ascertain the responsiveness of the data system to requests by Branch and Institute staff. The integration of the DRG system represented a major step forward prior to the study. It is anticipated that the integration of the two data systems will greatly assist the Branch Chief and staff in executing their analytical and statistical responsibilities.

The Conference Assistant position was abolished early in the fiscal year. The operating functions of this position were placed with conference program sponsors while the coordination of the conferences for fiscal and reporting purposes was added to the duties of the Administrative Officer.

The Committee Management Assistant provided various services to the nine advisory committees located in the Institute, including member processing

services. The National Advisory Child Health and Human Development Council served through the three councils constituting fiscal year 1974 with only eight appointed members of a total authorized membership of fourteen, not counting three liaison members. The Institute anticipates that five members will be appointed to the Council by June 30, 1974.

NICHD ANNUAL REPORT
JULY 1, 1973 THRU JUNE 30, 1974
GRANTS AND CONTRACTS MANAGEMENT BRANCH

SUMMARY

During Fiscal Year 1974, the Institute should support about 1,732 research and training grants, research fellowships, research career and research career development awards and research contracts at approximately \$118.4 million. Of this total, about 258 applications in the amount of \$16.1 million will be funded from Fiscal Year 1973 impounded funds which were released for obligation in February 1974.

The total number of grants awarded represents an increase of about 28% in the numbers of projects (385) and an increase of 32% in dollars (\$28.8 million) from the Fiscal Year 1973 portfolio.

The Institute used the DRG system of normalized scores to determine a funding order for regular research grants. Program projects were ranked according to an Institute-developed system for normalizing scores based upon the DRG formula. The priority score was essentially the basis for funding the training grants, research fellowship awards, and research career development awards, although preference was given to competing renewal training grants in order to provide continuous support to our ongoing programs. Competing training grants, postdoctoral and special fellowships (F2's and F3's) and research career development awards were funded from Fiscal Year 1973 impounded funds. The new individual research fellowship awards (F22's) were obligated from funds provided for in a separate line item in the Fiscal Year 1974 budget. Competing contracts were awarded from both the 1974 budget and the impounded funds.

Negotiation of Research and Training Grants

Noncompeting research grants with July 1 and August 1 start dates were not negotiated in Fiscal Year 1974. Those noncompeting grants with start dates of September 1 through February 1 were negotiated on a scale ranging from 5% to 15% depending upon the size of the grant. The March 1 through June 1 noncompeting grants had been negotiated in Fiscal Year 1973 at which time all future commitments were reduced. Competing research grants were negotiated on a similar scale of 5% to 15%. Through these negotiations we expect to acquire approximately \$4.2 million which will enable us to fund about 63 additional grants. No negotiation is anticipated for noncompeting training grants in Fiscal Year 1974.

RESEARCH GRANTS

During Fiscal Year 1974, we expect to award 1,120 regular research grants in the amount of \$74.9 million, an increase of 241 grants and \$19.8 million from Fiscal Year 1973. The total consists of approximately 608 noncompeting grants for \$40.5 million and 512 competing awards for \$34.4 million. Of the 1,120 awards, about 174 will be paid from impounded funds in the amount of \$8.6 million. About \$0.3 million of the \$34.4 million will be obligated for

scientific evaluation grants. In our Special Research Program we will award 11 Mental Retardation Center grants for \$5.6 million. In the Fiscal Year 1974 budget the research career and research career development awards appear as a separate line item under Special Research. We expect to award 92 research career and research career development awards in the amount of \$2.3 million. The total consists of 7 research career awards for \$0.2 million and 85 research career development awards for \$2.1 million. Nine of the research career development awards were competing applications paid from 1973 impounded funds (\$0.2). The total number awarded represents a decrease of 14 awards and \$0.3 million from Fiscal Year 1973. We transferred \$2.9 million to the Division of Research Resources for the General Research Support Grant program.

TRAINING GRANTS

During Fiscal Year 1974 we expect to award 100 training grants in the amount of \$12.5 million. We will award 66 noncompeting grants for about \$7.4 million. The balance of \$4.7 million represents impounded funds which were obligated to restore about 90 grants awarded in Fiscal Year 1973 to their required levels and to award about 34 competing training grants. The total number of grants increased over Fiscal Year 1973 by 7 and the dollar amount increased by \$5.6 million.

FELLOWSHIPS

We expect to award about 40 postdoctoral and special fellowships in the amount of \$0.5 million during Fiscal Year 1974. About 15 of these fellowships will be noncompeting continuations in the amount of \$0.2 million. The balance of \$0.3 in this program will be obligated to support about 25 competing awards.

NEW RESEARCH TRAINING FELLOWSHIP PROGRAM

A new individual research training fellowship program was initiated in Fiscal Year 1974. The NICHD was apportioned \$1.8 million to support this activity. We estimate these funds will support about 135 approved applications.

RESEARCH CONTRACTS

During Fiscal Year 1974 we expect to award 234 research contracts and reimbursable agreements for approximately \$20.8 million. This will consist of about 190 research contracts and reimbursable agreements in the amount of \$15.5 million for the Center for Population Research Program, about 14 contracts in the amount of \$0.8 million for the Adult Development and Aging Program, and approximately 30 research contracts in the amount of \$4.5 million for the Child Health Program areas. The Fiscal Year 1974 contract program will represent an increase of 20 contracts and reimbursable agreements and \$1.9 million from the Fiscal Year 1973 program.

SUMMARY OF INSTITUTE SUPPORTED PROJECTS
(Dollars in Millions)

| | 1973 | | 1974 | | Change | |
|-----------------------------|------------|-------------|------------|-------------|-------------|---------------|
| | No. | Amount | No. | Amount | No. | Amount |
| <u>Research Grants</u> | | | | | | |
| Noncompeting | 630 | \$40.3 | 618 | \$ 45.5 | - 12 | +\$ 5.2 |
| Competing | <u>260</u> | <u>20.4</u> | <u>513</u> | <u>35.0</u> | <u>+253</u> | <u>+ 14.6</u> |
| | 890 | 60.7 | 1,131 | 80.5 | +241 | + 19.8 |
| <u>Training Grants</u> | | | | | | |
| Noncompeting | 85 | 6.3 | 66 | 7.4 | - 19 | + 1.1 |
| Competing | <u>8</u> | <u>0.6</u> | <u>34</u> | <u>5.1</u> | <u>+ 26</u> | <u>+ 4.5</u> |
| | 93 | 6.9 | 100 | 12.5 | + 7 | + 5.6 |
| <u>Fellowships</u> | 44 | .5 | 40 | .5 | - 4 | 0 |
| <u>New Research</u> | | | | | | |
| <u>Training Fellowships</u> | -- | -- | 135 | 1.8 | +135 | + 1.8 |
| <u>Research Career and</u> | | | | | | |
| <u>Research Career</u> | | | | | | |
| <u>Development Awards</u> | 106 | 2.6 | 92 | 2.3 | - 14 | - 0.3 |
| <u>Contracts</u> | <u>214</u> | <u>18.9</u> | <u>234</u> | <u>20.8</u> | <u>+ 20</u> | <u>+ 1.9</u> |
| GRAND TOTAL | 1,347 | \$89.6 | 1,732 | \$118.4 | +385 | +\$28.8 |

NICHD Annual Report
July 1, 1973 through June 30, 1974
Program Statistics and Analysis Branch

SUMMARY

Much of the work of the Branch revolved around the preparation of reports for the use of program staff, the NACHHD Council, and the scientific community generally. Several publications resulted from these activities. Recipients of Research Grants from NICHD, to be published in Pediatrics, was based on a presentation made by the Chief of the Branch at a meeting of the NACHHD Council, and prepared in the Statistical Analysis Section. This paper analyzes the extent to which age, sex, and type of degree affect an applicant's chances of funding. The Program Analysis Section was responsible for the publication The Extramural Program of Research on Aging in NICHD, which includes information on the objectives and methodology of projects in aging research, as well as abstracts of current research progress. The Inventory of Federal Population Research, which covers all research grants, contracts, and intramural projects in population research supported by Federal agencies, was prepared by the Program Analysis Section, with the tabular material and the statistical analysis provided by the Statistical Analysis Section.

At the request of the Interagency Committee on Population Research, the Branch has undertaken to publish a comparable inventory of private agency population research. Discussions have been held with the major agencies in this field, who have agreed to provide the information in the required form. The data will be edited and classified by the Program Analysis Section and the computer aspects will be handled by The Population Council on contract.

A major Branch effort this fiscal year was planning for the integration of the DRG machine record system with the PSAB data system in order to avoid duplication of coding procedures and release time for analytical reporting. The Information Systems Section is working with the other Sections, and with DRG and DCRT, towards the implementation of the new data set-up.

The Branch is well represented in EEO activities. One of our members received an HEW award for outstanding contributions to the EEO program. Another is an NIH EEO Counselor and the NICHD representative to the NIH EEO Council. A third is a member of the NICHD EEO Advisory Committee. Several staff members are active participants in the NICHD Women's Task Force, serving on a number of the Task Force Committees.

Program Analysis Section

Activities in the Program Analysis Section remained unchanged in the last year, although there was expansion in some areas. The group continued to assign NICHD applications to the various programs within the Institute, to classify the grant holdings across a number of axes, and to prepare reports in answer to specific requests.

In the classification of grants, we have started coding intramural projects for the first time, as we have had an increasing number of requests for

covering information on research conducted as well as supported by NICHD. Another change has been the classification of the subprojects of program projects in order to provide better information retrieval for these large studies. In time we hope to get both of these additions computerized. In the meantime, we have devised a means for indicating on the computer whether a particular topic is of major or minor emphasis in a given project, which cuts down significantly on the amount of staff time spent in answering routine requests.

Several requests came to us as the direct result of Congressional interest in various areas of research. One of these was child abuse and/or neglect. Section staff were asked to prepare various status reports on NICHD funding of projects related to this topic, and also participated in planning sessions for the development of an NICHD conference on the subject. Other requests which were a reflection of interest on the Hill included those for information on Tay-Sachs Disease, Down's Syndrome, dyslexia, and abortion.

The Office of the Director also generated a number of requests in the course of the year. One of these was on developmental pharmacology; staff were asked to review NICHD applications in this area, and to categorize them according to a scheme developed by the National Academy of Science. Another covered Institute holdings in the fields of genetics, nutrition, and congenital malformations. Information on NICHD projects concerned with human sterilization and in vitro human fertilization was also requested by this office, as was an identification of the ten scientific disciplines which received the largest amount of support from NICHD.

As in the past, a good deal of the work of the Section involved jobs done to meet the needs of program staff. In connection with PBIM's preparation of a report to the NACHHD Council, Section staff reviewed applications and grants in the program with relevance for areas of special emphasis identified by PBIM staff. Also for incorporation in a report made by the GD program to the NACHHD Council, an analysis was made by research area, of all grants in the program since the Institute began, showing the changes that had taken place in the program over the 10-year period.

Another job in which the Section was involved with program staff concerned changes in the PR and GD program categories. PAS worked with staff in the two programs in developing the criteria for the new categories, and in subsequently assigning grants to them. In connection with Program Review, Section staff met with Perinatal Biology and Infant Mortality program staff in order to develop criteria for, and then to identify, all grants concerned with clinical trials. Other requests from program staff included identification of grants in genetics, parenteral nutrition, perinatal research supported by private agencies, oral steroid contraceptives, and low birth weight infants and the respiratory distress syndrome.

The Section continued to prepare quite a number of recurring annual reports for various purposes. Two of these are publications--the Inventory of Federal Population Research, and The Extramural Program of Research on Aging in NICHD.

Other annual reports prepared by the Section include the following: Scientific and Technical Information and Funds for Science for the National Science

Foundation; Reading, Communication, and Related Areas; Nutrition; Sudden Infant Death Syndrome; Sickle Cell Anemia; and Cooley's Anemia. The Section Chief continues to serve as one of the Institute's representatives on the Interagency Panel on Early Childhood and the Interagency Panel on Adolescence; material is also prepared annually for these panels on NICHD support of projects relevant to their respective interests.

Statistical Analysis Section

This Section participated during the year in the advance planning and preliminary work required for the contemplated switchover to machine input drawn from the DRG IMPAC system, which is expected to replace a large portion of the coding and data input preparation now done in the Statistical Analysis Section.

The Section continued to be responsible for the preparation and dissemination of a number of recurring reports, as follows:

- Annual: NICHD grants and contracts awarded from FY 1973 funds, alphabetic by investigator
 - NICHD grants and contracts awarded from FY 1973 funds, geographic by grantee location
 - NICHD grants and contracts awarded from FY 1973 funds, numeric by grant number
 - NICHD portion of NSF Survey of Federal Funds for Research, Development, and other Scientific Activities, Fiscal Years 1973-1975
 - Estimated distribution of NICHD intramural funds, by program, FY 1973
 - Vital statistics and other background data for the Director's use at appropriation hearings
- Semiannual: NICHD research contracts awarded from FY 1973 or active in June (December) 1973
- Quarterly: PBIM active grants and contracts, by program category
- Triannual: Status report on SIDS research
 - Active NICHD grants and contracts, alphabetic by Investigator
 - Active NICHD grants and contracts, geographic by grantee location
 - Active NICHD grants and contracts, numeric by grant number

Active NICHD fellowships, by program

A copy of the March 1974 Monthly Summary of Active Grants and Contracts is shown on page CB-6. (March data are the most recent available at this writing.) The Institute's active portfolio consists of 1431 grants and contracts totaling over \$102 million. These figures include 25 research grants totaling \$1½ million which had been funded and activated on FY 1973 impounded funds up to that point.

Statistical data bearing on the relationship between research project grants, program projects, and center grants was furnished to the NACHHD Council Subcommittee on Program Projects and Centers for each of its two meetings, in January and in April.

The Section also prepared charts, tables, and analytical narrative for the Adult Development and Aging Branch and for the Growth and Development Branch for inclusion in their respective reports to Council.

Reports provided to meet special needs of Institute staff included:

Summaries of review actions, approval rates, and funding rates for specific categories of grant applications

Trend data on grant and contract awards for specific programs

Vital statistics data of the National Center for Health Statistics

Data on NICHD indirect trainees and estimates of full-time-equivalent trainees

Biomedical/Behavioral distributions of NICHD grants and contracts

M.D./Ph.D. distribution of NICHD fellows and trainees

NICHD grantees who have received prizes or honors for research

NICHD awards to specific academic departments

Research funding success of investigators at MR Centers

Types of institutions receiving awards

Length of project period of PO1's

Fate of amended research grant applications

Organizations outside of NICHD to which the Section furnished information included Congressional committees, other NIH Institutes, and the Ford Foundation.

Publications:

LaVeck, G.D., Freedman, L.R., Walter, H.H., and Steinberg, F.S.:
Recipients of research grants from NICHD; do age, sex, type of
degree affect funding chances? Pediatrics, in press.

Information Systems Section

Major emphasis was placed on the planning, design, and programming for input linkage between the DRG IMPAC system and the PSAB machine data system. All Sections of the Branch were involved in developing the record content. A review of the current record content resulted in the release of some data elements no longer in use, and the adoption of new elements which will enhance the information the Branch can provide. The Section will operate on a parallel file update basis until the bugs are completely eliminated from the new system.

The Section has the responsibility for the computer aspects and publication preparation for the Inventory of Federal Population Research and The Extramural Program of Research on Aging in NICHD. A new task taken on by the Section this year was the machine preparation of the research abstracts for The Extramural Program of Research on Aging in NICHD. Working with the Program Analysis Section, a procedure was developed whereby the IBM conversational terminal and the Wylbur Text Edit System were utilized for input, and the IBM 360/20 remote terminal computer printer provided camera ready copy.

This operational mode not only saved time and money in producing the finished copy for the publication, but it also established a data set for use next year. Next year we will merely add new items to the file, remove items no longer active, and update the remaining items with the latest progress information. This will considerably reduce the time it takes to produce the body of this report for printing.

The Section developed and installed an improved fix program for the master file update system. The new program, written in PL/1, is less restrictive on input, executes in a faster fashion, and pulls forward more information.

NICHD Grants and Contracts Active During March 1974
(including scientific evaluation grants)

| Mechanism of Support and Category of Funds | Total | PR | ADA | Funds (thousands) | | | Child Health | | |
|--|-----------|----------|---------|-------------------|----------|----------|--------------|----|--|
| | | | | Total | PBIM | GD | GD | MR | |
| Research Grants - Total | \$ 64,532 | \$19,719 | \$6,097 | \$38,266 | \$11,732 | \$12,269 | \$14,265 | | |
| 1973 Impounded Funds | 1,294 | 248 | 26 | 1,020 | 499 | 424 | 98 | | |
| 1974 Congressional Increase | 225 | 225 | - | - | - | - | - | | |
| All Other | 63,012 | 19,245 | 6,071 | 37,245 | 11,233 | 11,845 | 14,167 | | |
| Training Grants | 7,670 | 1,504 | 1,503 | 4,663 | 1,624 | 1,899 | 1,140 | | |
| Fellowships | 420 | 85 | 54 | 281 | 138 | 112 | 31 | | |
| RCP Awards | 2,259 | 663 | 62 | 1,533 | 545 | 692 | 295 | | |
| Total Grants | 74,881 | 21,971 | 7,716 | 44,743 | 14,039 | 14,973 | 15,731 | | |
| 1973 Impounded Funds | 1,294 | 248 | 26 | 1,020 | 499 | 424 | 98 | | |
| 1974 Congressional Increase | 225 | 225 | - | - | - | - | - | | |
| All Other | 73,361 | 21,497 | 7,690 | 43,723 | 13,541 | 14,549 | 15,633 | | |
| Contracts (by related program) | 27,163 | 21,906 | 988 | 4,269 | 1,174 | 1,034 | 2,062 | | |
| Total Grants and Contracts | 102,044 | 43,877 | 8,704 | 49,013 | 15,214 | 16,006 | 17,793 | | |
| 1973 Impounded Funds | 1,294 | 248 | 26 | 1,020 | 499 | 424 | 98 | | |
| 1974 Congressional Increase | 225 | 225 | - | - | - | - | - | | |
| All Other | 100,525 | 43,403 | 8,679 | 47,992 | 14,715 | 15,582 | 17,695 | | |
| Number of Projects | | | | | | | | | |
| Research Grants - Total | 944 | 333 | 66 | 543 | 235 | 218 | 90 | | |
| 1973 Impounded Funds | 25 | 6 | 1 | 18 | 7 | 8 | 3 | | |
| 1974 Congressional Increase | 1 | 1 | - | - | - | - | - | | |
| All Other | 918 | 326 | 65 | 525 | 228 | 210 | 87 | | |
| Training Grants | 102 | 18 | 16 | 68 | 23 | 30 | 15 | | |
| Fellowships | 38 | 9 | 3 | 26 | 11 | 12 | 3 | | |
| RCP Awards | 92 | 28 | 2 | 62 | 22 | 28 | 12 | | |
| Total Grants | 1,176 | 388 | 87 | 699 | 291 | 288 | 120 | | |
| 1973 Impounded Funds | 25 | 6 | 1 | 18 | 7 | 8 | 3 | | |
| 1974 Congressional Increase | 1 | 1 | - | - | - | - | - | | |
| All Other | 1,150 | 381 | 86 | 681 | 284 | 280 | 117 | | |
| Contracts (by related program) | 255 | 206 | 16 | 33 | 9 | 7 | 17 | | |
| Total Grants and Contracts | 1,431 | 594 | 103 | 732 | 300 | 295 | 137 | | |
| 1973 Impounded Funds | 25 | 6 | 1 | 18 | 7 | 8 | 3 | | |
| 1974 Congressional Increase | 1 | 1 | - | - | - | - | - | | |
| All Other | 1,405 | 587 | 102 | 714 | 293 | 287 | 134 | | |

Notes: 1) Research grant totals include two scientific evaluation grants totaling \$450,776 not assigned to a program.

2) Excluded from all totals are 16 grants totaling \$594,750 and 2 contracts totaling \$154,450 that are funded by other Institutes.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Center for Population Research
Office of the Director

Introduction: Now in its sixth year as an organizational entity responsible for research on population issues, the Center can identify accomplishments and opportunities for rapid growth. The following examples illustrate CPR progress in providing essential tools to investigators.

1. Contraceptive Development Branch:

- Distribution of human reproductive hormones - standardized samples of human chorionic gonadotropin and its subunits (hCG) and of luteinizing releasing factor (LRF) are being supplied to the scientific community. These materials, produced by CDB contracts, were once scarce or unavailable as research material. In addition, distribution of ovine luteinizing hormone (LH) and its subunits will be initiated following their proper characterization.

- Testing Facility - The capability during the past year to test almost 200 compounds for antifertility and endocrine activities provides an invaluable resource to contractors in our antifertility drug synthesis program. In addition to its obvious function of testing specific compounds, this facility is enhancing the techniques and methodology of antifertility assays. Baseline studies on 16 reference standards have been completed and are being used in assays for potency. And, sophisticated improvements have been made in tests for postcoital antifertility and antispermatogenic activity.

2. Behavioral Sciences Branch:

- Support techniques for use of Census Public Use Samples - Each decennial census of the United States produces a wealth of data which is available to the public for analysis or other use to characterize the composition, distribution, and economic status of the American people. Because of its volume and variety, such data have sometimes proved unusable. Through a contract with DUALabs, the BSB has published a users manual for Census Public Use Samples (PUSH) to investigators. In addition, a CENTS-AID manual will be published which allows investigators to use the Public Use Samples with the fast cross-tabulation program (CENTS) initiated by the Census Bureau. Also, the contractor offers seminars and instruction in the use of both PUSH and CENTS-AID.

3. - Laboratory Test for Intravascular Coagulation - In clinical and investigative medicine, and with particular need for evaluating relationships between contraceptive use and thromboembolism, there existed an urgent requirement for laboratory tests capable of detecting or predicting intravascular coagulation. Research supported in this

area has resulted in development and standardization of procedures and methodology of fibrinogen chromatography as a screening procedure for assessing the risk of thromboembolic complications in patients on various types of contraceptive therapy.

4. Scientific Information Services:

- Population Sciences: Index of Biomedical Research - Since October 1973, the Center has produced this monthly bibliographic citation journal in cooperation with the National Library of Medicine based on information in the Library's Medical Literature Analysis and Retrieval System (MEDLARS). This resource is directed to investigators concerned with biomedical research in the population field. Distributed gratis during the first year, the journal will be available on a subscription basis with the September 1974 issue.

The observation that opportunities are accumulating for rapid growth is based in large part on estimates of dollar costs to move to the next step in contraceptive development - clinical trials. The testing in humans of new anti-fertility compounds is an expensive and complicated process which requires significant increases of budget and staff.

Budget: The fiscal picture for the Center is complicated in FY 74 by the same factors which affect most DHEW activities: the lack of an appropriation in FY 73; the impoundment of FY 73 funds which were subsequently released in FY 74; and a FY 74 appropriation from which the Congress permitted a 5% withholding. For simplicity, this report will reflect only the total amounts available for obligation in FY 74 in the various subject categories without accounting for differences in source whether from impounded funds, Congressional increase or appropriation base.

| | (Dollars in Millions) | |
|---|-----------------------|------------|
| | FY 1973 | FY 1974 |
| | (Actual) | (Estimate) |
| CPR Component -- Program Activity | | |
| 1. Population and Reproduction Grants Branch | (20.1) | (30.4) |
| A. Fundamental Biomedical Research | 12.8 | 19.4 |
| B. Fundamental Social Research | 2.6 | 3.5 |
| C. Population Centers | 2.6 | 4.0 |
| D. Research Training | 2.1 | 3.5 |
| 2. Contraceptive Development Branch | (6.5) | (6.8) |
| A. Directed Biomedical Research | 3.7 | 2.5 |
| B. Product Development | 2.8 | 4.3 |
| 3. Fertility Regulating Methods Evaluation Branch | (6.4) | (4.8) |
| Evaluation of Contraceptives in Use | 6.4 | 4.8 |
| 4. Behavioral Sciences Branch | (1.7) | (3.7) |
| Directed Social Research | 1.7 | 3.7 |
| 5. Office of the Director | (2.4) | (2.8) |
| A. Scientific Information Services | 0.1 | 0.2 |
| B. Population Program Management | 2.3 | 2.6 |
| 6. NICHD Intramural Research | (2.7) | (2.8) |
| TOTAL - Population Research Program | 39.8 | 51.3 |

As required by Public Law 91-572, (The Family Planning Services and Population Research Act of 1970) the Center submitted to the Deputy Assistant Secretary for Population Affairs the third annual progress report of population research programs for calendar year 1973. The report was prepared in late September and was subsequently submitted to the Congress by the Department. An expanded version of the final report was distributed in November by the Center to grantees and contractors funded by the Center.

Staffing: Adequate manpower to staff the programs of the Center became even more critical during FY 1974. Stringent personnel ceiling applied to the Institute resulted in the loss of one position to an authorized strength of 36 full-time permanent positions. The staffing pattern provides 22 professional and 14 clerical positions. Staff changes during the year included the following:

Behavioral Sciences Branch - Gloria Kamenske, Ph.D., was detailed to the Center from the Office of International Health, DHEW in July 1973 and was appointed to the staff in April 1974. Dr. Kamenske is a psychologist and received her doctorate from Michigan State University. She brings a rich background of Federal administrative experience to the Center from a variety of research and scientific management positions. Aaron Botbyl, Ph.D., resigned in July 1973 to return to clinical psychology work at the Cumberland County Guidance Center in Millville, New Jersey.

Population and Reproduction Grants Branch - Julia Lobotsky, M.S., was appointed at the end of June 1973 as a biologist. She was previously employed as a research scientist with the Worcester Foundation for Experimental Biology in Shrewsbury, Massachusetts. While with the Foundation, Ms. Lobotsky edited a scientific newsletter dealing with research in prostaglandins and conducted research in reproductive biology. William Spillane, Ph.D., Medical Social Worker, left the Branch and NIH to accept a position with the Drug Abuse Information Branch of the Alcohol, Drug Abuse and Mental Health Administration. Allyn Waterman, Ph.D., continues to provide valuable service to the Branch in an intermittent position as Health Scientist Administrator.

Fertility Regulating Methods Evaluation Branch - Appointment action was completed in May 1974 of Heinz W. Berendes, M.D., as Chief of this Branch. Dr. Berendes had been acting in that capacity for approximately one year. Donald B. Loveland, M.S., Statistician, retired on March 1, 1974. Mr. Loveland remains in Walnut Creek, California where his duties had been connected with the contract for the Contraceptive Drug Study at the Kaiser Foundation Research Institute.

Contraceptive Development Branch - Hyun K. Kim, Ph.D., received permanent appointment to the Branch as Health Scientist Administrator in May 1974. Dr. Kim is a medicinal chemist and held appointment with the Branch for the two previous years as a Senior Staff Fellow.

Liaison and Scientific Information: These activities are presented in this portion of the report since they are coordinated in the Office of the Director with substantial support from the Branches.

Interagency Committee on Population Research - The Center continues to provide staff support to the coordination of population research activities on the part of the various Federal agencies carried out through the Interagency Committee on Population Research (ICPR). An important function of this Committee is to facilitate the exchange of information and ideas and coordinate the efforts of the Federal Government concerned with research related to human population problems. The Committee has representatives from 20 Federal agencies and is chaired by the Director of the Center for Population Research. The Chairman was designated by the Deputy Assistant Secretary for Population Affairs, to whom the Committee reports.

Inventory of Federal Population Research - Fiscal Year 1973 - The ICPR issued its Inventory of Federal Population Research - Fiscal Year 1973, which is the fifth of such reports, updated annually. The Inventory represents a cooperative effort on the part of each of the reporting Federal agencies which funds projects in population research. The scope of the Inventory encompasses a broad spectrum of research in the biological, medical, social, and behavioral sciences and covers all projects--grants and contracts, intramural and extramural--in which population research constitutes a major substantive category according to the principal focus of the research.

Analysis of Federal Population Research - Fiscal Year 1973 - Another significant product of the ICPR is the Analysis of Federal Population Research - Fiscal Year 1973. This report, which is prepared annually by the Committee and submitted at the end of each fiscal year, fulfills an assigned function of the ICPR, namely, to make recommendations on actions to be taken to meet research gaps in the population sciences. It contains a summary and evaluation of current Federally supported population research with recommendations for needed research, based on the projects reported for the Inventory of Federal Population Research.

Population Sciences: Index of Biomedical Research - The communication of population research information is enhanced through the publication of Population Sciences: Index of Biomedical Research. Approval was received to publish the journal on a monthly basis, and periodic publication began in October 1973. This bibliographic citation journal provides a useful resource to those concerned with the progress and results of biomedical research in the population field. It is the counterpart of Population Index, a bibliographic journal devoted to population research in the social sciences, which the Office of Population Research at Princeton University publishes and which NICHD has helped support for several years. Population Sciences: Index of Biomedical Research is produced with the cooperation and assistance of the National Library of Medicine and is based on the information contained in the Library's Medical Literature Analysis and Retrieval System (MEDLARS).

Population Research Monographs - NICHD has initiated another important means of communication through its occasional monographs in population research. The monographs provide a review and evaluation of the state-of-the-art in substantive areas of population research. They are published as individual books on a non-periodic basis. The books usually contain an evaluative review of a field, annotated citations, and an analysis of the next steps for achieving progress with regard to meeting research gaps.

Publications of Workshops and Conferences - A number of publications have resulted from the proceedings and summaries of NICHD conferences and workshops in the population sciences. A list follows of the articles and books published this past year.

| Title & Year of Publication | Authors/Editors Publisher |
|---|---|
| Biology of the Cervix 1973 | R.J. Blandau K. Moghissi University of Chicago Press |
| Receptors for Reproductive Hormones (Scientific Symposium for the Annual Meeting of Population Research Centers Directors, 1972) 1973 | Bert W. O'Malley Anthony R. Means Plenum Press New York |
| The Corpus Luteum (Symposium of the Annual Meeting of the Society for the Study of Reproduction, 1972) 1973 | Gilbert S. Greenwald <u>Biology of Reproduction</u> (Supplement) |
| Biorhythms and Human Reproduction 1973 | Michel Ferin Franz Halbert Ralph Richart Raymond VandeWiele John Wiley-Interscience |
| Proceedings of a Research Conference on Natural Family Planning 1973 | William A. Uricchio Mary Kay Williams The Human Life Foundation Washington, D.C. |
| Oral Contraceptives and High Blood Pressure 1974 | Melvin S. Fregly, Ph.D. Marilyn S. Fregly, Ph.D. Dolphin Press Gainesville, Florida |

Staff Honors, Lectures and Articles:

Philip A. Corfman, Director

1. "Organized Population Research: Progress and Problems" Journal of Reproductive Fertility, 37:499-504, 1974. Publication of a paper presented to the annual meeting of the Society for the Study of Fertility, Reading, England, June 1972.

2. "Population Research at the NIH: A Response to a National and International Problem," a lecture presented at the Department of Obstetrics and Gynecology, the Medical College of Barcelona, Spain, May 30, 1973.
3. "Coordinated Studies of the Effects of Oral Contraceptives," paper delivered to the VIIth World Congress of Obstetrics and Gynecology, Moscow, U.S.S.R., August 16, 1973. Published in abbreviated form in Contraception 9, February 1974.
4. "Strategy for Contraceptive Development," Chapter 21 in Human Reproduction: Conception and Contraception, edited by E.S.E. Hafez and T.N. Evans, published by Harper & Row, Publishers, Inc., Hagerstown, Maryland, 1973.

Arthur A. Campbell, Deputy Director

1. "Beyond the Demographic Transition," Presidential Address to the Population Association of America, New York, New York, April 19, 1974, to be published in Demography, October 1974.
2. "Three Generations of Parents," Family Planning Perspectives, 5:106-112, 1973.
3. "Population: The Search for Solutions in the Behavioral Sciences," American Journal of Obstetrics and Gynecology, 116: 131-152, 1973.
4. "Fertility Patterns and Population Trends," Chapter 1 in Human Reproduction: Conception and Contraception, edited by E.S.E. Hafez and T.N. Evans, published by Harper & Row, Publishers, Inc., Hagerstown, Maryland, 1973.

Rolf Versteeg, Program Liaison Officer

1. Association for Population/Family Planning Libraries and Information Centers (APLIC), Member of the Board of Directors; Chairman, Training Programs Committee; Member, International Activities Committee; Member, Conference Planning Committee.
2. Population Association of America (PAA), Organized session for the 1974 PAA Annual Meeting on "Sharing International Population/Family Planning Information: Future Directions."
3. "Coordination and Communication Activities of NICHD'S CPR," Published in the May 1974 issue of Smithsonian's Science Newsletter.

William A. Sadler, Chief, Population and Reproduction Grants Branch

1. Guest Speaker, Dedication Ceremony of the Population Research Center, Baylor College of Medicine, Houston, Texas, November 1973.
2. "NIH Supported Population Research," talk as guest speaker to the Retreat of the Biomedical Center for Population Research of the University of Chicago Ben May Laboratory for Cancer Research, December 1973.

3. Lectures on Female and Male Reproduction, April 9 and 10, 1974 to the Department of Physiology and Biophysics, College of Medicine, Howard University, Washington, D.C.

Sidney H. Newman, Behavioral Sciences Administrator

1. American Psychological Association, Division of Population Psychology, Member of Executive Committee, and Secretary-Treasurer.
2. American Psychological Association, Annual Convention, September 1973, Montreal, Canada.
 - Chairman, Symposium on Problems of Human Crowding: Theory, Findings and Needed Research.
 - Member, Symposium on Problems of Sterilization
3. "Abortion: Needed Behavioral-Social Research." In Osofsky, Howard J. and Osofsky, Joy D., Editors. The Abortion Experience: Psychological and Medical Impact, 551-566, Harper & Row, Hagerstown, Maryland, 1973.

Kenneth Savard, Visiting Scientist

1. "In Vitro Observation on Follicular Steroids," presented to the University of Maryland, Physiology Department Seminar Series, January 30, 1974.
2. "Possible Role of Cervix in Contraception," presented to Symposium on National Family Planning, University of Florida School of Medicine, March 14, 1974.

Gabriel Bialy, Health Scientist Administrator

"Exploration of the Male Antifertility Programs," lecture presented at American University, Department of Biology, Washington, D.C., March 20, 1974.

Dolores J. Patanelli, Biologist

"Alternatives in Male Contraception," presented to Symposium on Men and Family Planning sponsored by Preterm, Washington, D.C., February 25, 1974.

Wendy Baldwin, Staff Fellow

Appointed in April 1974 to Editorial Board of "Contraception," a national journal based in Los Angeles, California.

Gloria Kamenske, Psychologist

Appointed to membership committee of the new Division of Population Psychology, American Psychological Association.

Heinz Berendes, Chief, Fertility Regulating Methods Evaluation Branch

1. "Maternal Health and Fetal Development" paper presented to the Symposium on the Development of the Mammalian Fetus in Detroit, Michigan, December 1973.
2. "Epidemiology of Perinatal Injury," paper presented to the Symposium on the Preventability of Perinatal Injury, New York, New York, March 1974.
3. "Perinatal Factors and Neurological Damage of Infancy" and, "Usefulness of the Apgar Score," lectures presented as a member of the guest faculty for a Continuation Course for Obstetricians and Pediatricians sponsored by the University of Nebraska, Omaha, March 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Center for Population Research

Research relating to population examines the interrelationships between population size, growth and distribution and economic development and other social and psychological factors, and analyzes ways in which individuals are motivated to reduce the size of their families. It is, therefore, concerned with a great variety of processes. It takes into account individual, interactional, familial, institutional, and social processes of great complexity involved in decisions relating to family size and the effective implementation of population policies and their consequences. Research in this area has been greatly expanded in line with NICHD's designation as the focal point for DHEW research in population.

Ad hoc advisory panels were established in FY 69 to guide the development of a program of research and to evaluate research proposals which are developed in the behavioral sciences in four areas:

- (1) The first of these areas of population research is concerned with the antecedents, processes and consequences of population structure, distribution, and change, and the interrelationships between social and population change. Special importance is attached to the interrelationships between economic factors and population growth, structure and distribution for this and other nations and for particular subgroups of the nation. Of increasing concern is the ecological impact of population growth and of related urban technological and industrial development, information about which is needed to guide the formulation of policies with reference to environmental quality. Attention is also being given to migration as a major vehicle of social change.
- (2) Major concern focuses on trends in fertility and related variables, such as marriage rates, age at marriage, unintended pregnancies, the incidence of abortion, trends in divorce, and changes in attitudes toward childbearing, family size, methods of fertility control, and other topics, including the failure to prevent unwanted births despite verbal commitment to family planning. Considerable effort is being made to develop a broader data base for more rapid determination of trends and the factors affecting them.
- (3) Ultimately the factors affecting trends in fertility will express themselves mainly within the framework of the family, so that a major area of research on population must concentrate on the family, on differences in this institution among various population groups, and on changes which are occurring in its structure, particularly as they affect fertility. Emphasis must also be placed on sexual behavior, both in and out of marriage, insofar as this bears on fertility. Research is also sought on the process of socialization

for marriage and parenthood, including the social pressures exerted by the immediate family, kin networks, peers, and the broader society to have--or refrain from having--children. More needs to be known about the factors influencing decisions that lead to the number and spacing of births, and including perceptions of alternatives to childbearing. Needed, too, is research on the relationship between family structure, childbearing patterns, and child development.

- (4) Concern with problems of population growth leads to questions of public policy. Research on all aspects of growth is thus relevant to policy but is also needed specifically on the ways by which population growth or movement are influenced by public policy, and the effects upon population of policies already adopted regarding family planning programs, welfare payments, parity payments, tax exemptions and allowances, direct incentives, etc. As to the relationship with family planning programs, the Center for Population Research is concerned with research on the assumptions under which programs are organized and the evaluation of these programs in terms of those assumptions: operational research is considered the primary responsibility of other agencies.

The above areas were included in Requests for Proposals from FY 69 to February 1972. They continue to reflect the scope of the behavioral sciences contract program in population research, but RFPs, based on workshops of experts, have shifted from covering the range of areas to targeting on specific areas pertaining to the determinants and the regulation of fertility, since fertility is the single most dynamic factor in population growth, and to the consequences of population growth and change as a guide to the formulation of public policy. Thus RFPs have been issued on "The Social and Economic Determinants of Fertility Trends in the United States, 1940-1970," "The Planning and Regulation of Fertility," "The Consequences of Childbearing and Child-spacing Patterns," and "The Economic and Environmental Consequences of Population Change."

A BSR Contract Review Committee was established in FY 74 to offer advice on the scope of the program and to evaluate research proposals. In this review function BSR-CRC members are supplemented by ad hoc review panel members.

About one-in-five of the proposals submitted under the contract program in the behavioral and social sciences have been approved by peer review committees and funded. Funding of approved projects totalled \$0.7 million in fiscal 1969, doubled to almost \$1.6 million in fiscal 1970 and redoubled to approximately \$3.9 million in fiscal 1971, dropped off slightly to \$2.9 million in fiscal 1972 and further to \$1.7 million in 1973, primarily due to delays in obtaining OMB clearance, but rose in 1974 to \$3.7 million, of which \$0.5 million in impounded funds can be carried over into 1975. Since the initiation of this research program in early 1969 through fiscal year 1973 a total of 77 contracts have been funded. During the current fiscal year a total of 11 contracts have been approved for funding, most of which will be funded during the current fiscal year. The remaining contracts will be funded in early fiscal year 1975.

Investigators in a wide variety of institutions, mainly universities, but also in governmental and private research organizations, have become involved in the research program. Fields of specialization represented are sociology, psychology, economics, anthropology, medicine, and statistics.

Since the beginning of the Behavioral Sciences Research program, several studies have been successfully terminated, but most are still in the active stage. As those now underway and others to be initiated are completed, it is hoped that our knowledge of the influences upon the birth rate and completed family size will be greatly expanded.

Much of the discussion about "the population problem" assumes that population plays a large role in many of our other social problems, but to what extent, and in what ways, are not sufficiently well known or agreed upon by competent scholars. Better knowledge concerning the effects of population growth, structure, and distribution are essential if informed and acceptable public policies regarding population are to be formulated and adopted. These studies, dealing with a variety of interrelated factors not easily disentangled, require that a program be structured in long-range terms, and it will be at least several years before results highly useful to policy makers will emerge.

Emphasis here is on projects which were continued (including those for which a final report was received) or funded and initiated in fiscal 1974.

During this fiscal year 62 contracts were active (including 3 reimbursable agreements with other Federal agencies), 21 of which are extensions (with additional funds) of projects negotiated in previous years.

Socio-economic Correlates of Fertility

Twelve studies deal with socio-economic correlates of fertility in various historical and cultural settings.

The Princeton University (DA-16) 1970 National Fertility Study, funded by NICHD as was the 1965 study, is the latest in a series of studies since 1955 that have attempted to document the changes in family size preferences of American women, and other factors affecting their fertility. The 1970 data reveal an increase in the use of the most effective methods of fertility control, an increase in the percentage of the population approving abortion, and a drop in the rate of unwanted fertility between the early and late 1960s. The increasing popularity of the pill, the IUD, sterilization, and abortion means that more people will require a physician to meet their family planning needs. The National Fertility Study has also shown that some of the historic differences in contraceptive use by religion were being eased as more Catholics used methods not sanctioned by their church; and low income couples, probably through the efforts of family planning programs, have almost caught up to the level of contraceptive protection experienced by high income couples.

A Georgetown University study (DA-17) is analyzing the fertility behavior (including age at marriage, family size aspirations, and contraceptive methods)

of a much earlier cohort of women (born 1901-1910) because they achieved the lowest fertility so far recorded in the United States.

To probe the courses of the rise and decline in fertility in the United States between 1940 and 1970, several studies are concentrating on an hypothesis advanced by Professor Richard Easterlin. Easterlin has argued that the rise in fertility in this period was due to the relative improvement in economic prospects for young people in the marriageable ages which led to earlier marriages, earlier childbearing, and an increase in family size. Economic prospects, according to this hypothesis, are perceived relative to the level of living enjoyed by young people in their families of origin. The decline since 1960, Easterlin states, has been due to a decline in the relative economic prospects of young people. The four studies subjecting this hypothesis to more rigorous empirical tests are indicated below.

The Population Studies Center at the University of Michigan (DA-18) is subjecting the Easterlin hypothesis to formal modeling and econometric testing and estimation using the 1940-1973 period of observed U.S. fertility as evidenced by data from the National Center for Health Statistics and from Current Population Surveys and decennial censuses.

The Institute of Social Research at Michigan (DA-19) is undertaking four studies with respect to the recent change in U.S. fertility as the dependent variable: (1) an examination of individual changes in fertility expectations between 1967 and 1973 using survey data from a panel of 500 households to evaluate how changes in employment status, income, and consumption activities relate to expressions of expected family size and actual reproduction; (2) analysis of the relationship between changes in female higher education and employment between 1955 and 1970 to changing fertility of cultural subgroups by using Growth of American Family and National Fertility Study data; (3) the possible relationship of the changing sense of economic "well-being" between 1952 and 1973 to fertility by using Survey Research Center consumer attitude survey data and fertility statistics; and (4) testing the Easterlin hypothesis by using new micro-level survey data on family income, history and consumption aspirations, and childbearing patterns.

Indiana University (DA-20) is initiating a series of empirical studies designed to increase our understanding of cohort and annual trends in fertility in the white and non-white populations over the period 1917-1972. A time series analysis of national data will distinguish between several components of the fertility rates (age, cohort and annual effects) and will investigate the relations between deviations from fertility trends and economic trends. This analysis will include relative status as an exploratory variable and will add controls (current economic conditions and simple income effects for young men) to allow an estimation of the net effect of relative income on fertility. The second approach will be an examination of the relative impact of the Easterlin income ratio when other independent variables (e.g., "marriage squeeze", military manpower, and school enrollment) are controlled. Third, "get along income" (as measured by Gallup Polls) will be substituted for the relative income status measure; and, fourth, female opportunity costs will be introduced in the analysis of fertility and female marital status.

Analyses will be extended to subgroups wherever possible. Finally, state data will be used to test the Easterlin hypothesis.

The Center for Policy Research (New York) study (DA-21) is using national fertility and census data to develop a causal model linking social, economic, and demographic variables related to U.S. natality trends since 1950. Major sets of independent variables to be used in the model are: (1) current economic conditions of young adults relative to expected conditions; (2) sex roles and female employment; (3) the age structure as reflected in marriage and employment opportunities; and (4) the availability of contraception and abortion.

Rand Corporation research (DA-22) identified the principal determinants--e.g., income, time of marriage, female labor force participation, and migration characteristics--of family size and current fertility patterns in the United States. The influence of socio-economic factors on fertility should provide the basis for estimating the probable impact on the birth rate of: (1) increased years of schooling, (2) unearned income supplements to the working poor, (3) change in tax deductions for dependents, (4) housing and rent subsidies, and so forth. The study finds some confirmation of the Easterlin "economic taste modification mechanisms" hypothesis, but finds the data analyzed also compatible with intergenerational transmission and persistence of frequencies regarding family size.

The Research Institute for the Study of Man (DA-23) has found that while fertility of Spanish-Americans has traditionally been higher than that for the total population of the United States, increasing education is decreasing fertility for these groups as well as for the general U.S. population. For high school graduates as of 1970 the fertility of Mexican origin women was only moderately above that for all white U.S. women, and the fertility of mainland-born women of Puerto Rican origin was actually equal to or below that for all U.S. white women. In addition, Mexican-American men have shown marked improvement in occupational composition in the past 40 years, and mainland-born Puerto Ricans are in the process of becoming indistinguishable from the U.S. population in occupational profile. These educational and occupational changes suggest a convergence with the fertility pattern of the general population in the decades ahead.

A major Princeton University study (DA-25) deals with the correlates of fertility reduction in European countries since the 19th century. Poorly educated rural populations reduced fertility by voluntary control, even when mortality was higher than it now is in less developed countries. Three broad conditions appear to be necessary for a decline in fertility: the acceptance of calculated choice as a valid element in marital fertility; the perception of the advantages to be gained from reduced fertility; and knowledge and mastery of effective techniques of control. Apparently modernization ultimately establishes these conditions, but they can occur--and did occur--in communities with very little modernization.

The objective of research undertaken at Western Washington State College (DA-26) was to investigate social, political, and economic influences bearing

on fertility, including rural differentials by size of land holding and abortion and fertility differentials by employment status of women in Poland, and the socio-economic correlates of fertility and birth control in the U.S.S.R.

A Food Research Institute, Stanford University, study (DA-28) covering a number of developing countries has revealed that the demographic transition from high to low rates of population growth is occurring on a broad scale, but the speed with which it is proceeding, and the socio-economic circumstances (including urbanization and education) having the greatest impact upon it varies from country to country. Peasant communities in Indonesia, Nepal, and Peru have been studied in the field by Columbia University (DA-29) investigators to assess the costs and benefits associated with different family sizes in each community. In Indonesia the perception that children can do chores at relatively young ages that free the mother to engage in economic activity is a factor in maintaining high fertility. It has been observed elsewhere that compulsory school attendance may reduce the usefulness of children.

Sex Roles and Socio-Psychological Correlates of Fertility

Research underway in Massachusetts by the Worcester Foundation for Experimental Biology (DA-30) is testing attitudinal factors affecting family size. Women who see a career as a source of personal gratification may desire a small number of children so that their family interests do not prohibit them from continuing their interests in a career. "Competent" (rational, practical, independent, etc.) women had smaller completed families in the middle and lower socio-economic levels but larger families at the highest SES level.

Several studies are investigating the relation of fertility to marriage and family structure. The Institute for Survey Research, Temple University, (DA-32) is studying the role of husband-wife communication and decision-making regarding fertility-related activities. In the preliminary phase of this study analysis of group interviews shows that some couples plan the number and spacing of children and contraceptive usage before or soon after marriage while other couples discuss these matters only after economic and other pressures of too many children are felt. Husbands appear to be more interested than their wives in the sex composition of the family--wanting either a son or both sons and daughters. The decision-making process regarding contraception is an initial decision to do something, followed by a health professional's recommendation (to the wife) of a method, and another contact if the first method is not satisfactory. The study will test these preliminary impressions more extensively.

An Indiana University study (DA-33) found that role "modernity" emerged as a stronger predictor of birth intentions among young married couples than did race, religion, or socio-economic status. The less traditional that couples are in terms of their definitions of appropriate husband-wife behavioral patterns, the fewer children they intend to have. Higher status males appear to be more motivated than lower status men toward having fewer children both because of their own orientation toward individualism and their acceptance of their wives' individualistic interests; it is speculated that lower status men may want more children because they obtain fewer gratifications from their occupations. These

data suggest that empathy--satisfaction with husband-wife communication and understanding--is related to the setting of agreeable fertility goals and the successful practice of contraception.

The University of California at Los Angeles (DA-35) has been awarded a contract to elucidate the behaviors and motivations of 120 employed career women relating to fertility and family planning and to specify the patterns of temporal ordering of family and work. The Center for Policy Research (DA-36) has undertaken a study of occupational experience as a determinant of fertility among American women. The principal investigator is examining an important aspect of the relationship between women's participation in the labor force and their fertility behavior, namely, the characteristics of the occupations they work in. This inquiry is particularly directed toward determining what effect on fertility is resulting from the increasing number of women who are taking or attempting to take a more active role in the labor market, especially in professional occupations. Emory University (DA-37) is focusing on the influence of childbearing on women's labor force participation by analyzing the 1960 and 1970 Census Public Use Samples.

A study based on a complex model of the relationship between fertility and women's labor force activity, initiated at Research Triangle Institute, N.C. (DA-38), suggests that antecedent social and economic variables determine labor force participation and fertility and that labor force participation affects fertility only indirectly. A wife's preference for work has little effect on the timing of the first conception but may delay--and rule out--the possibility of a third pregnancy. Couples who expect to be more affluent appear to make less effort than other couples to postpone the first conception, but those who are in fact more affluent are likely to experience a later first conception. The data also show that women who find outside childcare acceptable have earlier first pregnancies than those who do not.

A Brown University study (DA-40) found that participation of women in the labor force and higher levels of literacy and education have contributed to declines in fertility in a developing country (Thailand).

The University of Kansas (DA-42) is investigating the dynamics of social norms concerning large and small families--reactions to explicit and implicit social pressures in relation to normatively defined family size ideals.

A Calspan Corporation study (DA-43) has isolated six specific values which influence the preferred number of children: economic concerns in general and providing for children in particular; parental attention to each child; companionship of siblings; the mental strains and worries of parenthood; physical work and energy requirements of parenthood; and over-population concerns. These factors are negatively correlated with desired family size indicating that the significant fertility values are those that deter people from having large families rather than those that encourage them to have many children. The anticipated "costs" of having children, rather than the anticipation of rewards, constituted the major determinant of desired family size. Drastic differences in correlations between those who had no children and those who had one suggested that the arrival of the first child has a profound effect

on the perception of family size consequences. Yet "values," especially emotional satisfactions, of having children were positively correlated with couples who had one child and, to a lesser extent, with those who had four children.

Wright Institute (California) analysis of data on 3,180 Vassar College women during the period 1954 through 1960 confirmed the following hypotheses: (1) in those years almost every Vassar student aspired to marriage and to motherhood; (2) almost no student expressed interest in adoption unless a physical condition hampered pregnancy; and (3) almost no student expressed concern with problems of overpopulation. In addition, it was found that two-thirds of the women wanted careers, which they expected to interrupt for child-rearing and then to resume. Most were content with the status of educated women in the 1950s as evidenced by the fact that seniors desired an average of 3.4 children. Sexual attitudes and behavior became considerably more liberal during the college years.

The Center for Policy Research (DA-45) has completed a research project to examine the possible relationship between ideology and fertility-related behavior. Specifically, the study included the development of a quantitative model which will examine the relationships between: (1) family background, (2) family ideology (the attitudes and values of the respondent's mother and father), (3) the respondent's ideology, (4) the respondent's career and family role orientations, (5) sexual attitudes, (6) sexual behavior, and (7) preferred family size. A contract with Illinois State University (DA-46) will investigate the influence of attitudes towards sexual behavior held during teenage years on fertility attitudes and behavior during the early years of family formation.

Part of the study of the consequences of childbearing is to focus on the consequences to the child. Two longitudinal studies are underway which have this focus. In a College of William and Mary study (DA-47) age at marriage and time to first birth are the major independent variables. It is hypothesized that these variables will be related to family interrelationships, parental attitudes and children's personality development. In the Princeton University Educational Testing Service Study (DA-48) it is hypothesized that birth order, socio-economic status, and the sex of the infant will be related to mother-infant interaction and to cognitive and emotional development of infants. A Calspan Corporation study (DA-49) has found a small but highly reliable inverse correlation between intelligence of offspring and family size--even when socio-economic status and intelligence of parents are taken into account. While theory on the process by which family size can produce psychological effects is meager, a dominant explanation of family size effects seems to lie in the diminution of interaction with parents as family size increases and to result in diminished verbal development, which in turn has an effect on operative intelligence.

A University of Wisconsin study (DA-51) based on data from the 1967 Survey of Economic Opportunity found that the rate of marital disruption of first marriages is twice as high for Blacks as for Whites under age 45--24% vs. 12%. Of those separating, only 60% of Blacks but 88% of Whites get divorced--a fact attributable in part to differential access to the legal procedures for ob-

taining a divorce. Early marriage is more closely associated with marital disruption than any other factor, e.g., a white woman marrying before age 18 has a 50% higher probability of ending her marriage in separation or divorce than if she had waited to marry. But for those whose marriages are terminated, remarriage is likely, especially for men, for persons under age 40, and for women with fewer than two children. For Blacks, the lower rate of remarriage and the much higher incidence of widowhood is more important in explaining the large number of black families headed by women than is the incidence of marital disruption.

Research underway at Bowling Green State University (DA-53) involves a re-interview of mothers interviewed in Toledo in 1963 coupled with a sample of married women and their husbands in the Toledo SMSA to test for possible associations, within broad religious and socio-economic categories, between selected social-psychological and demographic variables. A positive association has been found between high alienation scores, lower socio-economic class status, early marriage and high incidence of premarital and early pregnancies. High alienation scores in 1963 were associated with higher levels of fertility over the next eight years and with higher proportions of women who wanted no additional children nonetheless. Alienation scores remained relatively constant over the eight-year interval.

Demographic and Attitudinal Factors Affecting Family Formation

Research being undertaken in Louisville, Kentucky, by the Human Resources Research Organization (DA-55), is exploring the factors involved in "moral judgment" with respect to marital status and family size. This study assumes that moral judgments are key factors in social pressures to conform, that single people and childless couples are widely viewed as blameworthy, and that this is deleterious to population control efforts.

Another study, being undertaken at the University of Southern California (DA-56), looks to the kin network in the United States as the locus of influence for its members with respect to fertility and migration decisions.

A University of Kansas (DA-57) study, surveying 1,000 first-time parents classified on the basis of the extent to which such factors as goal orientation, traditionalism, and economic aspirations affected their decision as to the number and spacing of births, is being expanded to determine the work patterns and childcare arrangements of the women after the birth of their first child.

Fertility, Abortion, Illegitimacy and Family Planning

Center for Population Research contracts indicate both the concentration of interest in fertility and family planning and the range of problems dealt with. The Census Bureau (DA-59), under a reimbursable agreement, has used the Current Population Survey as a vehicle for studying the relationship between age at marriage, birth intervals, and total fertility. The most notable

finding has been the decline in the number of births expected by married women aged 18-24. In 1973 as in 1972, the average number of births expected by such women was 2.3, compared with 2.9 in 1967 and 3.1 in 1965. The proportion of young married women expecting a total of two children increased from 37% in 1967 to 56-57% in 1972 and 1973, while the proportion expecting three or more children declined from 56% to about 30% in 1972 and 1973. The number of children expected by young women is now consistent with the replacement level of fertility--that is just enough births to replace the number of people in the parent generation. If such low rates continue indefinitely, the population of the United States will stop increasing, except for immigration, in about 65 years.

The Census Bureau, under another reimbursable agreement, (DA-60) is analyzing data on current fertility, 1960-1969, together with data from a special tape file dealing with women aged 14-54, including information where applicable on husbands, children, and other household or family characteristics. The purpose is to provide data by socio-economic characteristics for several population groups on: (1) age at marriage, intervals between marriage and first birth, and birth intervals for later births by cohort to determine any changes in childspacing patterns from cohort to cohort; (2) the effect on fertility of separation and of marital dissolution and remarriage; (3) unintended child-bearing; and (4) social and work roles of women.

DUALabs (DA-61) has developed a support technique to facilitate the wider use of the 1960 and 1970 Census Public Use Samples (PUS). The project emphasis is on the handling of very large data files by writing programs to merge different types of records, create smaller work tapes and provide programs for cross tabulations and analysis of the data. DUALabs has completed work on the PUSH software system, which enables researchers to use readily available computer analysis programs (such as the Statistical Package for the Social Sciences, SPSS) with the Public Use Sample. The PUSH users manual was published by GPO and is available through NICHD; the tape is being distributed by DUALabs. In addition, DUALabs has developed a CENTS-AID program to allow investigators to use the Public Use Samples with the very fast cross-tabulation program (CENTS) initiated by the Census Bureau. The CENTS-AID manual is being published by GPO and will be distributed in the same manner as the PUSH manual. DUALabs is also offering seminars and instruction in the use of both PUSH and CENTS-AID.

A University of Georgia study (DA-62), analyzing the trends and patterns of population change for the total white and non-white populations of all counties in the United States from 1950 to 1960, concentrated on fertility and migration. This study, completed last year, found that whites in predominantly Spanish-American counties and nonwhites in American Indian counties had the highest crude birth and total fertility rates of whites and nonwhites, respectively, in the five major types of counties. The traditionally high fertility of the Appalachian Region had disappeared by 1950-1960, for both the white and non-white residents of this area had the lowest crude birth and total fertility rates of the five major ethnic or cultural groups. Regression analyses revealed that, other than dominant cultural group, the age structure of counties was the most important explanatory variable in accounting for total, white and nonwhite crude birth and total fertility rates.

A study on the consequences of illegitimacy, and methodological problems associated with studying the phenomenon, was completed at the University of California (Berkeley) last year. That study found that total fertility has declined more among black than among white women, the apparent result of the more frequent use of abortion among blacks. That black women use abortion more frequently while achieving higher total fertility suggests that they use contraception less frequently and less effectively and have more unwanted pregnancies. Legal abortions in California have reduced illegitimate births even more than legitimate births. This study has also shown that the rate of illegitimacy, especially for white women, leveled off in the most recent year studied, actually rising for white women under age 20, while legitimate births have continued to decline. This study is being followed up by one at the same institution looking at the interrelationship of illegitimacy, abortion and family structure variables, including patterns of sexual relationships (DA-64).

An essentially methodological study, arranged under contract with the University of North Carolina (DA-65), seeks to ascertain through computer simulation the effects on fertility of various childspacing patterns in an effort to improve the analysis of current trends in fertility. This project has been broadened to include: (a) a comparative study of the sensitivity of alternative indices to changes in fertility, emphasizing indices based on birth intervals as compared with the more traditional measures of total fertility and age specific fertility rates, and (b) development of documentation of the POPREP simulation model of the general biological model of population growth and the associated computer program SURVEY used for simulation of sample surveys. A Temple University study (DA-66) is designed to assess physicians' current perceptions, understandings, and attitudes toward birth control methods which may be considered as possible abortifacients.

The CPR behavioral science role with respect to family planning programs in the U.S. and abroad has been identified by advisory groups and staff as relating on the micro level to individual motivation for commencing and continuing with family planning services and on the macro level to the demographic impact of family planning programs. Funded studies reflect this role.

A University of North Carolina (UNC) (DA-67) sample field study concentrated in low income areas of 16 cities is measuring the impact of family planning services on contraceptive practices and reproductive experience by comparing data for cities where adequate family planning services are available with cities with less adequate services. Another UNC study (DA-68) builds upon several aspects of that household survey to test over a three-year period a utility model of fertility designed to predict the probability of various reproductive events to couples on the premise that individuals regulate their reproductive behavior in the light of their expectations and perceptions of the consequences of reproduction for them. Variables include perceived consequences and measures of costs and rewards of facilitating or preventing a subsequent birth.

A study being carried out by the Center for Family Planning Program Development (DA-69) is attempting to determine the effects of family planning programs on the fertility of women throughout the United States.

Under a contract with the University of North Carolina, (DA-70) a study is planned in Tamil Nadu, India, to investigate whether there is a threshold of social and economic development after which family planning action programs will make more rapid progress. A study at the University of Colorado (DA-71) is looking into the relationship between social mobility within urban areas and the acceptance of family planning services.

Studies undertaken by Bowman Gray School of Medicine, Wake Forest University (DA-72) among blacks in New Jersey and North Carolina and among white and Indian women in Costa Rica show that: (1) blacks have accepted the national family size ideal but evidence a wide range of acceptable number of children around that ideal point; (2) in Costa Rica consensual unions, contrary to the literature on Caribbean blacks, do not depress fertility; (3) women in consensual unions differ from those in legal unions primarily in terms of social class and related KAP (Knowledge, Attitude, and Practice) factors; and (4) psychological factors may be of greater importance in determining family planning clinic attendance than previously reported in the literature. i.e., fears and untoward experiences with clinics and contraceptives are more important than a woman's demographic characteristics in predicting whether or not she will continue attending a clinic.

Migration, Social Mobility, and Residential Patterns

Ten contracts deal with migration. The University of California (DA-74) found in an analysis of 1969 OEO data for 1800 lower socio-economic respondents that most migrants had increased their income and viewed urban opportunities more favorably than rural opportunities although one-third desired to return to a rural area. Most of the migrants had considered only one destination--and that only a short time before migrating--had moved only where relatives or friends already resided, and had a job lead. One-fourth of rural interviewees were considering migration, but the preference was clearly for remaining in-state. Most non-migrants tended to be older, married, property owners, participants in government assistance programs, and to have more children and dependents. At the same time the study explored the phenomenon that many of the rural poor may be "trapped" by their poverty or may value their membership in a rural social community more than the potential improvement to be gained through migration. Those most critical of rural opportunities had the lowest education, income and employment prospects, and more family responsibilities and other ties (or binds) to the rural area.

The relationship between migration perceptions and experiences was generally similar for the three migration streams studied. "Appalachians", however, tended to migrate out of economic necessity, prefer the rural lifestyle, and consider returning to the rural area. "Pull" factors and concern for amenities were more dominant for Blacks, and discrimination problems were more salient to Southeast Blacks than to Southwest Spanish-Americans.

Policy recommendations designed to alter or redirect the urban migration of the rural poor include the improvement of opportunities for employment and services in the rural area and the facilitation of commuting or migration to regional growth centers.

A completed University of Kentucky study of migrants from Eastern Kentucky indicated that migrants generally increase their income and the community (Cincinnati) derives far more benefits from the migrants than they cost the city. Many of the successful migrants would nonetheless return to Kentucky at lower wages, but are deterred from doing so because the communities from which they came cannot provide sufficient inducements. The investigators concluded that these areas can be viewed within a single social system, since the economic and social fluctuations in one area have a noticeable effect on the welfare of the other and that policies designed to reduce concentration in cities must give greater attention to balanced economic development and job creation in areas where manpower is already available.

Another study dealt with the economic costs and benefits of migration to areas which lose and areas which gain from migration. The study, undertaken at Colorado State University, (DA-76) was concerned with the population changes that occurred during the period 1950-1970--about half of the counties have been losing population--in the Mountain Region stretching from Montana to Arizona and considered the consequences of those changes to public finances, retail activity, housing utilization, and agricultural activity.

TRACOR, Inc. (DA-77) has prepared a draft of "Rural-Urban Migration in the United States: An Annotated Bibliography and Synthesis of Research" for publication by the Government Printing Office as a "CPP" (Center for Population Research) monograph in FY 1975.

Battelle Laboratories, Columbus, Ohio (DA-78) conducted an analysis of migratory responses to employment opportunities. The aim of this research was to clarify the relationship between migration and certain demographic and economic variables. Migration was found to be responsive to employment opportunities, with college-educated men tending to move more frequently than the non-college-educated.

The U.S. Bureau of the Census under a reimbursable agreement, combined data from the March Current Population Survey taken by the Bureau for the years 1966-1970 to study migration during that period by single years of age in order to assess the joint influence of various socio-economic and familial statuses on the probability of making different types of moves during an individual's lifetime if he moved according to present age-specific rates of residential mobility.

Another Census Bureau study (DA-80), also under a reimbursable agreement, will explore how the absolute level of individual and family income and type of income interact with other variables to influence decisions to move, the distance moved and region of destination. It will also investigate such consequences of migration as effects on earnings, trends in income and fertility, and progress of children in school. To meet these objectives, the 1960 census data relating to migration will be retabulated to be compatible with tabulations available from the 1970 census and the Current Population Survey, 1961-1970.

A Brown University study (DA-81), based on a probability sample of women residing in Aberdeen, Scotland, who had at least one live birth during the period October 1950-September 1955, was designed to investigate: (1) how spacing of

pregnancy and of fertility varies; (2) the life conditions under which women are likely to resort to terminations and sterilizations; and (3) how the size of the family of origin and the position on a traditionalism scale are related to social mobility, present socio-economic status, aspirations for children, and fertility. High aspirations for one's self and one's children were found to bring about declines in fertility.

A University of Michigan (DA-82) study is analyzing 1970 U.S. census data on tapes for 20-25 SMSAs (Standard Metropolitan Statistical Areas) and is comparing these with census materials for four Canadian metropolitan areas to determine: (a) variations in population distribution in different metropolitan areas; (b) the degree of segregation of families in different stages of life cycle; (c) the degree of residential segregation of different socio-economic groups; (d) the pattern of segregation of housing of different types and ages; and (e) the relation to housing type to socio-economic characteristics of occupants. Preliminary findings indicate that in the 1960s Blacks continued migrating out of the South to the three other regions but at a slower pace. There was no substantial in-migration to the South, either of northern-born Blacks or of southern-born returnees. Inter-metropolitan migration of Blacks increased relative to rural-urban migration during the decade. Substantial out-migration of Whites from large central cities continued. In such places as Detroit, Cleveland, and St. Louis, net out-migration equalled one-third of the population present at the beginning of the decade. Several large cities--including Cleveland, New Orleans, and St. Louis--experienced a net out-migration of Blacks. Nonetheless, Blacks continued to be much more concentrated in the central cities than were Whites.

The University of Texas (DA-84) is studying the demography of the black population. The purpose of this study is to describe and analyze trends in the demographic characteristics of the black population, and compare these with trends in the white population. The basic analytical approach is cohort analysis by sex and color.

Consequences of Population Growth

One major study by the University of Virginia (DA-85) of the consequences of population change seeks to analyze those components of the Gross National Product (frequently used as an index of economic well being) which offset disadvantages of population growth (pollution, etc.) and, therefore, represent diseconomies. An early finding of this study is that while GNP increases more rapidly with more rapid population growth, per capita GNP rises more rapidly when population grows more slowly.

Studies are being undertaken to estimate the major effects on the economy, the resource base, and the environment of a number of alternative demographic developments, both nationally and regionally, that may occur during the next 30-50 years. One study by Resources for the Future (DA-86) is estimating what the results would be for the economy and the environment under alternative demographic conditions. The interrelationship between demographic change and employment growth in metropolitan areas, where 70% of the U.S. population lives,

is being examined by the Urban Institute.(DA-88)The theoretical and policy issues related to the consequences of metropolitan population stability or decline is being studied by Scientific Analysis Corporation.(DA-89)

Research Oriented Toward the Possible Development of Population Policies

A University of Illinois project, completed last year, explored the effect of income on fertility by asking hypothetical questions (both direct and projective) in a 1971 regional sample survey concerning the effect on personal fertility of government payments for more or fewer children. Projective questions indicated a greater responsiveness to incentive payments than did direct questions, but even the latter suggested that money incentives could have a decided effect on fertility behavior. Moreover, significantly higher proportions of respondents said they would have fewer children than more children at various levels of incentive payments. As anticipated, persons with higher incomes were less responsive to incentives than were those with lower incomes. This study was reported in Julian L. Simon's The Effects of Income on Fertility, Monograph 19, Carolina Population Center, University of North Carolina at Chapel Hill, 1974.

The Pennsylvania State University (DA-90) has been awarded a contract to investigate in detail, historically and contemporaneously, the non-metropolitan population changes in the State of Pennsylvania. That State is a large mature area with a highly diversified non-metropolitan sector. The investigators propose to create a data bank of relative historical-statistical information and to create a typology for the non-metropolitan areas in order to analyze the most important processes shaping change in those areas. Data will be derived primarily from the census but several other sources will be used and field studies will be conducted. The studies will focus on transport, technology, changing labor force roles of females, attitudes toward metropolitan living, and others-- which have shaped the non-metropolitan sectors of the population.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: National Fertility Survey, 1970-1975
Contractor: Princeton University
Money Allocated: \$212,260 (FY 1970); \$519,349 (FY 1971); \$390,871 (FY 1972);
\$165,699 (FY 1974)

Objectives: The objective is to survey approximately 6,500 women representing a national sample of all ever-married women under age 45 in the conterminous U.S. to determine fertility behavior and fertility expectations of American women. The study is a continuation of studies begun as the Growth of American Families Study, 1955, repeated in 1960, and of a similar National Fertility Survey conducted by the present investigators in 1965. The study compares fertility behavior and expectations by age, marital duration, race, education, income and religion, and will supply data on the use of various contraceptive methods, and the influence of a broad range of factors on their use. It will include a sample of women previously married but not currently in the married, husband-present category.

Major Findings: This survey has documented a substantial decline (36 percent) in the rate at which women were having unwanted children between the period 1961-65 and 1966-70. This improvement in the control of fertility resulted from increases in the proportions of couples using contraception and, particularly, in the proportions using the more effective methods. A time series on the use of oral contraceptives shows that the proportion of married women under age 35 using this method rose from about 2 percent in early 1961 and 30 percent in 1967 and remained close to this level through September 1970. The study also shows that the use of sterilization for the prevention of conception has become much more common than it was. Among older couples of childbearing age (wife aged 30-44) using any method to prevent conception, 25 percent report sterilization (12 percent of the husbands and 13 percent of the wives). In 1965 the comparable proportion was 16 percent. Sterilization has become the most common method of fertility control at the later childbearing ages and oral contraception is the next most common (21 percent).

Significance to Biomedical Research and Program of the Institute: This study will continue a unique series of studies on fertility, birth expectations and contraceptive practices among women in the U.S., and will provide a base of reference for prospective studies on contraceptive practices and the influences upon the health of women and children. It is an essential part of the public health aspect of the study of contraceptives as well as of attitudes toward family size.

Proposed Course: This contract, initiated in 1970, will be completed in fiscal 1975, with the publication of a book detailing findings of the study. A follow-up of the study to determine to what extent fertility behavior since 1970 is consistent with expectations in 1970 is contemplated.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of Low Fertility Cohorts in the United States

Contractor : Georgetown University
Money Allocated: \$99,911 (1973); \$16,140 (1974)

Objectives: This study seeks to gain a deeper understanding of the low fertility of the 1920's and 1930's in the United States, by studying the cohorts of women born during 1901-1910. These cohorts achieved the lowest fertility recorded to date in the demographic history of the United States. The proposed study is comprised of three complementary parts. First, an analysis of existing data relating to the fertility of the 1901-1910 cohorts. This involves data cleaning of the tapes from the Indianapolis Study, the first of the major U.S. fertility studies. Second, there will be a review of the demographic literature about the 1920's and 1930's, especially that which focuses on fertility trends. Third, there is preliminary work which will lead up to a sample survey of women born between 1901 and 1910. This aspect consists of pretesting items and developing techniques for interviewing these women.

Significance to Biomedical Research and Program of the Institute: As part of the Institute's interest in understanding the determinants and consequences of fertility, this project will fill a major gap in our understanding of how and why women restricted their fertility. The women under study had the lowest total fertility rates ever recorded in the U.S. and yet they accomplished this prior to the introduction of most "modern" contraceptives. The motivations of these women and the means they used to implement these motivations will bear on our understanding of the general issue of birth control and motivations for parenthood. By re-analyzing extant data the investigator will make the fullest possible use of available information before attempting to gather new data.

Proposed Course: This study has been funded for 18 months. If the investigator determines that the available data leave questions unanswered and that the women born 1901-1910 are interviewed, a larger field study may be undertaken.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : An Econometric Analysis of United States Fertility
Change, 1940 to 1973
Contractor : University of Michigan, Population Studies Center
Money Allocated : \$62,978 (1974)

Objectives: The purpose of this study is to subject the Easterlin hypothesis to formal modeling and econometric testing and estimation by using the 1940-1973 period of observed U.S. fertility as evidenced by data from the National Center for Health Statistics and from Current Population Surveys and decennial censuses. The project has four specific aims. The first is to model the effect of population size and age structure on relative income, which in turn is used to model fertility. In this regard, "different age groups of labor are viewed as imperfect substitutes in production," suggesting that "a larger cohort will thus tend to receive reduced age-specific incomes over its life cycle..." The second aim is to test the model using simultaneous equation methods and age-specific data on fertility, population, income and labor force participation in the United States over the period specified. The third aim is to "analyze the dynamic behavior of the estimation model...using spectral analysis techniques." The fourth aim is to develop fertility forecasts based on the estimated model. In this regard, the nature of the model permits confidence regions to be obtained.

Significance to Biomedical Research and Program of the Institute: This proposal is highly relevant to the testing of the Easterlin hypothesis requested in the RFP 74-9 concerning the "social and economic determinants of fertility in the United States, 1940-1970." The originality of the approach promises to make a very valuable contribution both substantively and methodologically. The approach is particularly suitable to the problem at hand, i.e., the elasticity-of-substitution relating to various age groups is ingenious, and the data are appropriate. It may contribute also to forecasting future fertility--a very important factor in biomedical research.

Proposed Course: This contract is funded for three years.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Fertility Change After the Baby Boom: The Role of
Economic Stress, Female Employment, and Education
Contractor : The University of Michigan, Institute for Social Research
Money Allocated : \$219,968 (1974)

Objectives: Undertake four studies with respect to the recent change in U.S. fertility as the dependent variable: (1) an examination of individual changes in fertility expectations between 1967 and 1973 using survey data from a panel of 500 households to evaluate how changes in employment status, income, and consumption activities relate to expressions of expected family size and actual reproduction; (2) analysis of the relationship between changes in female higher education and employment between 1955 and 1970 to changing fertility of cultural subgroups by using Growth of American Family and National Fertility Study data; (3) the possible relationship of the changing sense of economic "well-being" between 1952 and 1973 to fertility by using Survey Research Center consumer attitude survey data and fertility statistics; and (4) testing the Easterlin hypothesis by using new micro-level survey data on family income, history and consumption aspirations, and childbearing patterns.

Significance to Biomedical Research and Program of the Institute: The project is thoroughly relevant to RFP 74-9 concerned with "the social and economic determinants of fertility in the United States, 1940-1970." The underlying idea is important and original, and the results are likely to have considerable significance to expanding our understanding of the determinants of fertility.

Proposed Course: This contract is funded for two years, beginning in June 1974.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Impact of Economic, Demographic and Social Factors
on Fertility Trends in the United States, 1917-1972
Contractor : Indiana University
Money Allocated : \$126,393 (estimated, 1974)

Objectives: The purpose is to increase our understanding of cohort and annual trends in fertility in the white and non-white populations over the period 1917-1972. A time series analysis of national data will distinguish between several components of the fertility rates (age, cohort and annual effects) and will investigate the relations between deviations from fertility trends and economic trends. This analysis will include relative status as an exploratory variable and will add controls (current economic conditions and simple income effects for young men) to allow an estimation of the net effect of relative income on fertility. The second approach will be an examination of the relative impact of the Easterlin income ratio when other independent variables (e.g., "marriage squeeze," military manpower, and school enrollment) are controlled. Third, "get along income" (as measured by Gallup Polls) will be substituted for the relative income status measure; and, fourth, female opportunity costs will be introduced in the analysis of fertility and female marital status. Analyses will be extended to subgroups wherever possible. Finally, state data will be used to test the Easterlin hypotheses.

Significance to Biomedical Research and Program of the Institute: The testing of Easterlin's theory and alternatives to it is clearly relevant to RFP 74-8 concerning "the social and economic determinants of fertility in the United States, 1940-1970." The development of new measures is considered requisite to the proposed test.

Proposed Course: This contract is funded for two years.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Comparative Analysis of Economic, Age Structure and Sex Role Determinants of Recent American Natality Trends
Contractor : Center for Policy Research, New York
Money Allocated : \$59,458 (estimated, 1974)

Objectives: This multivariate analysis of American natality trends since 1950 will employ a causal model to link social, economic, and demographic variables. Major sets of independent variables to be used in the model are: (1) current economic conditions of young adults relative to expected conditions; (2) sex roles and female employment; (3) the age structure as reflected in marriage and employment opportunities; and (4) the availability of contraception and abortion. Basic data will be from the 1955 and 1960 GAF studies and the 1965 and 1970 National Fertility Studies. Additional data for "cohort variables" will be collected from census materials. Models will be constructed and data analyzed for each survey period. As a second stage, the data will be longitudinally analyzed. A final version of the causal model will be utilized to project natality estimates. A more restricted model (owing to data limitations) will be used to calculate reverse projections of natality.

Significance to Biomedical Research and Program of the Institute: This theoretically sound project should provide some evidence of the methodological significance of the multivariate causal analysis approach to analyzing fertility trends since 1950. It is therefore very relevant to population research in NICHD.

Proposed Course: This contract is funded for two years.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Investigation of the Determinants of Fertility in the United States
Contractor: The Rand Corporation
Money Allocated: \$160,900 (1971)

Objectives: This study attempts to identify the principal social determinants of family size and current fertility patterns in the United States. Using three sets of data (1) Survey of Economic Opportunity 1967, (2) Longitudinal Survey of Family Economics (1967-1970), and (3) a Time Budget Survey taken in 1964, the investigator is concentrating on the effects of such factors as income, time of marriage, female labor force participation, and the timing and frequency of migration upon size of family. The method of analysis consists of multivariate analysis of data from the three surveys.

Major Findings: The methodology developed in the course of this study offers some improvement in prediction of qualitative variations in demographic behavior, which should contribute to the analysis of fertility behavior and to improved estimates of how proposed reforms in the welfare and tax systems will influence family labor supply and fertility. The methodology does, however, require further examination. One portion of the study finds some confirmation of the Easterlin "economic taste modification mechanisms" hypothesis, but finds the data analyzed also compatible with intergenerational transmission and persistence of preferences regarding family size.

Significance to Biomedical Research and Program of the Institute: The study of the determinants of fertility and the development of methodologies related to them are one of the major concerns of the program in the Behavioral Sciences of the Center for Population Research. Through the process of multivariate analysis this study will clarify the effect of the various factors already known to affect fertility and will describe with greater precision the behavioral and biological mechanisms affecting fertility and how these differ for various segments of the U.S. population. The study should provide a basis for estimating the probable impact on the birth rate of (1) increased years of schooling, (2) unearned income supplements to the working poor, (3) change in female earnings relative to those of males, (4) change in tax deductions for dependents, and (5) housing and rent subsidies.

Proposed Course: This contract was funded in 1971 and was completed during fiscal 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of Fertility of Spanish-American Populations in the United States and their Socio-Economic Correlates

Contractor : Research Institute for the Study of Man, New York
Money Allocated: \$254,315 (1972)

Objectives: Using data from standard and special 1970 and earlier decennial census tabulations, the investigator is examining the fertility (and its socio-economic correlates) of the major Spanish-American population groups in the U.S.: Mexican, Puerto Rican, Cuban, Central and South American and the descendants of the original (17th and 18th Century) Spanish group. The 10 million Spanish Americans living in the United States comprise about 5% of the total U.S. population. Information on country of birth of the foreign born and the foreign-born parents of the native born, on mother tongue, and Spanish surname will be used with data on children ever born and such standard demographic items as age, sex, marital status, educational attainment, labor force and employment status, family income, etc. to ascertain fertility trends and differentials among these groups for as long a period as the data permit.

While encompassing an historical analysis, this study emphasizes the testing of several specific hypotheses: (1) factors associated with fertility differentials generally (urban-rural residence, educational attainment, labor force and employment status, occupations of the husband and wife, and family income) pertinent in kind, if not in degree, among the Spanish-American groups; (2) residence in ethnic concentrations results in higher fertility, and conversely dispersed living results in fertility approximating that of the general population; and (3) cultural continuity carries over from the country of origin but is diminished with each success of generation born in the U.S.

Significance to Biomedical Research and Program of the Institute: Diverse information on the Spanish-American population groups in the United States has suggested much higher fertility, somewhat higher mortality, and generally poorer health conditions than among the general population. This study is the first to attempt to look at the groups as a totality and at the similarities and dissimilarities among them. The most important groups are the Puerto Ricans concentrated in the New York City area, Mexican-Americans in the Southwest, and the Cubans primarily on the Eastern seaboard. From a policy point of view for family planning programs, etc. it is desirable to have baseline data as to the level and trends of fertility among the various Spanish-American population groups.

Major Findings During Year: A paper on "...the Fertility and Occupational Composition of the Mexican-American and Puerto Rican Origin Populations

Living on Mainland U.S." found that: (1) for both groups fertility decreased with increasing education and for high school graduates by 1970 the fertility of Mexican origin women was only moderately above that of all white U.S. women while the fertility of mainland-born women of Puerto Rican origin was actually equal to or below that for all white U.S. women; (2) among Puerto Ricans fertility was higher at each educational level for island-born than for mainland-born women; (3) fertility of Mexican origin women in the U.S., while higher than that for all white U.S. women, has decreased more than has fertility of women still living in Mexico; (4) occupation composition of Mexican-American men showed marked improvement between 1930 and 1970 but still lags a generation behind that of the total U.S. male population; (5) island-born Puerto Ricans are roughly on the same occupational level as the Mexican origin and Negro populations, but the mainland-born Puerto Ricans are--or within perhaps two decades will be--indistinguishable from the occupational profile of the general U.S. population.

Proposed Course: This contract, begun in 1972, is for a three-year period.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Analysis of the Decline of Fertility in Europe,
by Province

Contractor : Office of Population Research, Princeton University
Money Allocated: \$68,670 (FY 1970); \$87,160 (FY 1971); \$21,977 (FY 1972);
\$113,384 (FY 1973); \$49,603 (FY 1974)

Objectives: The purpose is to analyze the decline in fertility in the more than 700 provinces in Europe from the time in the 19th Century that fertility was essentially constant until the present, or until it reached a minimum and began to rise. Its aim is to determine the circumstances, in terms of the education, religion, occupational structure, residence patterns, etc., of the population, under which fertility declined. This study will make a definitive contribution to the social history of Europe and will substantially improve our understanding of the factors relating to the decline of fertility.

Major Findings: So far, two books have been published as a result of this project: A Century of Portuguese Fertility, by Massimo Livi Bacci, and The Decline of Fertility in Germany After 1870, by John Knodel (in press). In addition, a great deal of methodological development has occurred as a result of this study--particularly a new procedure for reconstructing age distributions on the basis of time series of births and deaths.

A recent finding of general interest relates to the effects of declines in infant mortality on the fertility rate. It has generally been supposed that a decline in infant mortality is a pre-condition for reductions in fertility--that is, reductions in infant mortality tend to increase average number of surviving children, and parents compensate for this rise by having fewer births. Knodel's manuscript on the decline of fertility in Germany shows that the decline in marital fertility and the decline in infant mortality proceeded at about the same time, but in almost half of the provinces the decline in fertility came before the decline in infant mortality.

Other findings suggest that the diffusion of knowledge about methods of fertility control may operate to reduce fertility independently of some of the social and economic factors often thought to be essential in accounting for reductions in fertility.

Significance to Biomedical Research and Program of the Institute: Fertility has been designated as an area of the highest priority by the Center for Population Research. This study will furnish information in far greater detail than has previously been available on the social and the economic factors associated with the decline in fertility in many countries of Europe.

Proposed Course: This project, underway for several years, was extended into fiscal 1974.

NIGHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Human Fertility in Poland and the U.S.S.R.

Contractor : Western Washington State College
Money Allocated: \$47,169 (1971); \$5,580 (1974)

Objectives: The primary objective is an investigation of social, political, and economic influences bearing on fertility in Poland and the U.S.S.R. and including rural fertility differentials by size of land holding, and abortion and fertility differentials by employment status of women in Poland, and the social and economic correlates of fertility and birth control in the U.S.S.R.

Since the mid-1950's the Soviet Union and the Eastern European countries within its sphere have published more demographic and economic data and have liberalized abortion laws (partly because of limited housing and the need to increase the number and proportion of females in the labor force).

The demographic processes in the Communist countries need to be studied for the light they may shed upon relationships of population to socio-economic-political conditions which are both similar to and different from those in other regions. Fertility differentials between the more developed parts of the RSFSR on the one hand and the five Union Republics of Central Asia and of the Caucasus, and of the autonomous republics on the other hand should throw additional light on basic demographic processes in various cultural contexts. In addition, the effects of sharp and contradictory policy changes need exploring both to assess the effects of such shifts and to avoid similar problems in the U.S.

Significance to Biomedical Research and Program of the Institute: All aspects of the research are program relevant, but particularly the impact of abortion, of urban-rural, religious and other ethnic differentials and, more generally, of "modernization," on fertility trends. Moreover, the investigator is one of a very small number of persons in the U.S. with both the language and technical skills to pursue studies in the Slavic countries.

Major Findings: The inverse relationship between human fertility and education is fully substantiated with data for the 36 major ethnic groups in the U.S.S.R. Variation in fertility attributable to the traditionally religious values can be explained in terms of the age-specific marriage and educational differentials known to have existed in the past and still present in the multi-national population of the Soviet Union.

A number of social and demographic variables indicate that fertility bears no relation to the proportion of economically dependent women in the political and social context of the Soviet Union. Non-fertility variables (disproportion between married women and married men and the percentage of urban

population, and other components identified with modernization, such as education, divorce rates, etc.) bear more significantly on female employment. The decrease in the marriage differential for men and women and the rise in urbanization between 1959 and 1970 suggests a substantial rise in the proportion of females not entering into, or retiring from, the labor force.

Proposed Course: This study initiated in 1970, has been extended to early FY 1975.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Socio-economic Factors in the Reduction of Natality
in the Less Developed Areas

Contractor : Food Research Institute
Stanford University

Money Allocated: \$60,902 (1970); \$65,698 (1971); \$84,402 (1972);
\$11,873 (1973)

Objectives: The objective is to examine the usefulness of the notion of the demographic transition, or modifications thereof, as a model for fertility reduction in non-Western areas, and to determine the context of socio-economic conditions that appear to be the basis for the onset of a decline in fertility in the underdeveloped or developing countries of the world. Measures of natality and of socio-economic development are being studied in each of the several major regions of the world to determine their accuracy and comparability, and to analyze their interdependence. The study will also seek to provide a possible measuring stick or projective device for estimating the effects of efforts to reduce fertility in the future.

Significance to Biomedical Research and Program of the Institute: This study will provide insight into the process of fertility decline in the developing and underdeveloped regions of the world and the extent to which the socio-economic conditions associated with natality decline in the West are having, or are likely to have, the same effect on the non-Western world, as well as indicating what additional factors may influence fertility decline in these areas.

Major Findings: This study, covering a number of developing countries, has revealed that the demographic transition is occurring on a broad scale, but that the speed with which it is taking place, and the socio-economic circumstances having the greatest impact upon it varies from country to country. These factors can be summarized generally however, as being related to increasing urbanization and education.

Proposed Course: This contract, begun in FY 1970, was renewed with funding for FY 1971 and was completed in FY 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Economic Cost and Value of Children in Four Societies

Contractor : Columbia University
Money Allocated: \$215,320 (1971)

Objectives: This research is directly aimed at the assessment of the importance of the economic costs and benefits of children as possible factors in fertility decisions. The secondary aim is the development of more refined research methodology for such studies.

Peasant communities in Indonesia, Nepal and Peru have been studied using anthropological techniques of intensive observation together with extensive questionnaire surveys. Field investigation and data analysis have been carried out by research assistants familiar with the particular communities in which they have done the research. Intensive observation of domestic and productive activities have been used to assess the costs and benefits associated with different family sizes in each community, and census and fertility surveys have been carried out to determine actual fertility and attitudes regarding fertility. Data analysis will provide comparable data for all societies.

Major Findings: In Indonesia the perception that children can do chores at relatively young ages that free the mother to engage in economic activity is a factor in maintaining high fertility.

Significance to Biomedical Research and Program of the Institute: This study deals with a crucial area of population research that has definite relevance to the Institute's program. Very little information has been available on the economic cost or benefit of children and how these are perceived, in either industrial or non-industrial societies.

Proposed Course: This study, initiated in 1971, is scheduled for completion in FY 1975.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Attitudinal Factors Affecting Family Size

Contractor : Worcester Foundation for Experimental Biology
Money Allocated: \$40,089 (1971); \$42,397 (1972); \$39,036 (1973);
\$19,000 (1974)

Objectives: This research (1) tests the hypothesis that women who perceive little difference between sex roles and who reject (for themselves) the stereotype of women as less assertive, ambitious, intelligent, etc., than men are the ones most likely to accept employment and to desire and achieve relatively small family size; and (2) the perceived sex-role differences and self-concepts will be related to antecedent biographical characteristics and family backgrounds. The second goal of the study will provide information for a later longitudinal study. The sample will include 1,000 persons--375 married men, their 375 wives, 125 single men and 125 single women of evenly distributed ages (17 to 55) and socio-economic levels (based on husband's occupation or, for younger men, education). The subjects will be obtained from local civic, professional, religious, and labor union groups and will be selected to be representative of the community.

Major Findings:

This analysis has indicated that there are at least two major and essentially unrelated dimensions of individual differences related to reproductive and career phenomena in women--a "competency-incompetency" and "warm-cold" dimension of self-concept.

The data analyzed at this point show that for Catholic women who have completed their childbearing, those who incorporate more of the positively valued masculine characteristics of "competency" (i.e. rational, practical, independent, etc.) had significantly smaller completed families than those women with more stereotypic "incompetent" self concepts. There are, however, complex interactions with social class. For all women (Catholic and non-Catholic) aged 45-54 the study found that the "competent" women had smaller families than "incompetent" women in the middle and lower socio-economic levels, with the reverse true at the highest socio-economic level.

The competency-incompetency dimension, while not related to the incidence of employment, appears to be related to the degree of interest in career, employment and attainment outside the family.

The warmth-expressiveness dimension measures the positive aspects of the female stereotype and includes attributes such as being emotional, talkative, able to devote self to others, and so forth. This dimension is not related to completed family size but is related to pregnancy planning and childspacing. The more a woman exhibits these characteristics the further apart she tends to space her children.

While these findings are based on a restricted sample, they offer insight into how personality characteristics may influence fertility behavior.

Significance to Biomedical Research and Program of the Institute: This study of several aspects of the relationship between achieved or planned fertility and the perception of the sex role of women has importance to the understanding of fertility behavior, which is crucial to the Institute's mission.

Proposed Course: This project is expected to be completed in fiscal 1975.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Study of Fertility-Related Decision-Making Among Married Couples
Contractor : Institute for Survey Research, Temple University
Money Allocated : \$120,369 (1973)

Objectives: The main objective of this study is to investigate the nature and etiology of decision-making and implementation processes related to fertility regulation of married couples. This will be accomplished by an interview study of a sample of 450 married couples living in urban, suburban and rural areas. Interviews will be conducted with both marital partners. The study will provide analyses of decision processes and related attitudes focusing first on the couple as a unit and then on both the husband and wife.

The study will investigate: (A) The degree of congruence of knowledge and attitudes among the husbands and wives, (B) the ability of each spouse to accurately perceive the attitudes of the other, (C) the amount and direction of communication between them on topics of fertility and fertility control. Planned analysis will focus special attention on the effects of, and disparity in, partner's attitudes upon the success of fertility control.

Major Findings: Preliminary analysis of group interviews shows a variety of approaches to fertility related decision-making. Some couples plan their number of children, spacing, and contraceptive use from the beginning, or even before marriage, and others discuss these things only after the pressures of too many children are felt. The pressures expressed by husbands are most often economic. Also it appears to be husbands who are more interested in the sex composition of the family, either wanting a son or wanting both sons and daughters. While wives are less concerned about this they seem to be aware of their husbands' wishes.

The decision-making process regarding what contraceptive is chosen appears to be an initial decision to do something, a recommendation to the wife from a health professional regarding a method; evaluations of the method by the couple; and possibly another contact with the health profession if the first method was not satisfactory.

Significance to Biomedical Research and Program of the Institute: Fertility decision-making, as an intervening factor between social and psychological attributes and fertility behavior is a topic relevant to the interests of the Center. This study will also make methodological contributions regarding techniques for interviewing men on fertility-related subjects.

Proposed Course: This is a one year contract during which time the survey instrument will be designed, the study carried out, and the data analyzed.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Sex Role, Family Structure, and Fertility Control
Contractor : Indiana University
Money Allocated : \$207,260 (1970); \$37,120 (1971)

Objectives: The purpose is to study the relationships between definitions and perceptions of male and female roles within the family and achieved and anticipated fertility. The specific concern of the study is with those decisions relating to childbearing and with the antecedent conditions that structured them, with emphasis upon the husband-wife interaction and, particularly, with reference to the alternative sets of roles and rewards which are perceived as valued and open to the female. Emphasis will be placed upon the effect of the wife's employment and career orientation upon decisions with respect to the size of family. The contract involves the interviewing of a regional probability sample of 3,000 persons, 15 to 45 years of age, drawn from the East North Central Region of the United States.

Major Findings: In this analysis role modernity emerged as a stronger predictor of birth intentions among young married couples than did race, religion or sex. The less traditional couples are in terms of their definitions of appropriate husband/wife behavior, the fewer children they intend to have. The more that wives adhere to and husbands concur with modifications of traditional husband behaviors requiring males to relinquish their long-standing prerogatives (allowing wives to gain greater individualistic gratifications) the fewer family-oriented rewards are intended. In seeking alternative rewards couples are motivated to avoid the costs of a greater number of children. Higher status males appear to be motivated toward having fewer children than lower status men both because of their own orientation toward individualism and their acceptance of their wives' individualistic interests and lower status men may want more children because they obtain fewer gratifications from their occupations.

These data also suggest that feelings of empathy--satisfaction with husband and wife communication and understanding--are related to reduced birth intentions. To the extent that empathy is a valued marital reward it tends to function as an alternative to familistic satisfactions. Furthermore, empathy seems to be related to the setting of agreeable fertility goals and the successful practice of contraception.

Significance to Biomedical Research and Program of the Institute: This contract bears on many aspects of the Institute's concern with contraceptive practice and family planning: age at marriage, decisions affecting fertility, alternatives to childbearing, such as employment of the woman, probability of divorce, and socialization for marriage and parenthood.

Proposed Course: This contract was initiated in FY 1970 at a funding level of \$207,260. It was renewed in FY 71 with \$37,120 in funds. The progress

of the project has been excellent, but unavoidable delays were experienced with the survey. The project will be completed in FY 1975.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Employed Woman: Family Planning and Careers

Contractor: University of California at Los Angeles

Money Allocated: \$38,361 (1972); \$34,820 (1974)

Objectives: The purpose of this proposal is to elucidate the behaviors and motivations of 120 employed career women relating to fertility and family planning and to specify the patterns of temporal ordering of family and work. Psychological motivations for having children will be delineated and the intensity of desire for children will be compared for each group of women with the intensity of desire for various alternatives to childbearing. One important facet of the research will consist of an attempt to quantify the perceived rewards and cost of both the motherhood role and the career role. Data analysis will be structured to relate reward cost data directly to fertility variables such as actual number of children and expected number of children and to career variables such as length of full-time employment, personality and developmental factors, that may influence the differences in employment history, fertility and desire for children.

Significance to Biomedical Research and Program of the Institute: This study represents an attempt to gain insight into the relation of family limitation and childbearing and work experience and aspirations of women.

Proposed Course: The contract initiated in 1972 to run for one year required an extension in time through FY 1974 to offset delays in obtaining OMB final clearance.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Occupational Experience as a Determinant of Fertility
Among American Women
Contractor : Center for Policy Research
Money Allocated : \$104,800 (1972)

Objectives: The negative relationship between women's participation in the labor force and their fertility is well established. Previous studies, however, have analyzed the relationship between women's working and fertility in terms of participation and non-participation. The proposed study will take a more sophisticated strategy by considering the characteristics of the work women do and the relationship between these occupational characteristics and fertility. The study's main goal is to determine whether differences in occupational opportunities and rewards result in variations in the fertility behavior of women.

The study will use published data from the 1940, 1950, 1960 and 1970 censuses. The bulk of the analysis will not be based on published material, but rather will be based on the one percent Public Use Sample tapes of the 1960 and 1970 censuses. The 1970 one percent tapes will be especially useful since the interview schedule contained a number of retrospective questions concerning the occupation of each household member in 1965. Moreover, there are a number of questions on the subject's marital history and number and age of children. Lastly, the tapes will have an occupational code which has a high level of specificity that will allow the researcher to categorize occupations in the most suitable theoretical and methodological manner.

Significance to Biomedical Research and Program of the Institute: This project will make valuable contribution to the population field. The principal investigator intends to examine an important aspect of the relationship between women's participation in the labor force and their fertility behavior, namely, the characteristics of the occupations they work in. Recently there are indications that a large number of women are taking or attempting to take a more active role in the labor market, especially with professional occupations. Currently, it is not known how the differential participation in the labor market or different sectors of the labor market influences fertility and social institutions, such as the family. This project would do much to better understand contemporary and future changes.

Proposed Course: This contract was initiated in FY 1971 and will run for a three year period.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of Child-Bearing and Labor Force Participation
of Women
Contractor : Emory University
Money Allocated : \$56,009 (1973)

Objectives: The main purpose of this study is to investigate the effect of fertility on labor force participation. The analysis will be based on Census data available through Public Use Samples of 1960 and 1970. The investigator will first seek to determine how the relationship between childbearing, childspacing, and labor force participation changed from 1960 to 1970. A series of control variables will serve to standardize biological and demographic factors and include but are not limited to such variables as age, age at first marriage, parity of the most recent birth, childspacing, family size, migration mobility status, marital status, and number of times married. The second stage of the research will focus on the influence of the other variables on the relationship between childbearing and employment, as for example, how economic need influences a woman's returning to work after the birth of a child.

Significance to Biomedical Research and Program of the Institute: This proposal deals with an area of considerable interest to the Institute, the relationship between women's labor force participation and fertility. This proposal emphasizes the influences of fertility on labor force participation. While there is undoubtedly a reciprocal relationship this study will focus on consequences of childbearing.

Proposed Course: This project was funded for one year with an expected renewal at the end of that year.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Branch

Contract Title : Women's Labor Force Participation and Fertility

Contractor : Research Triangle Institute
Money Allocated: \$109,692 (1971); \$4,318 (1974)

Objectives: This two-phase study is based on a complex causal model of the relationship between women's fertility and their labor force activity. The model suggests that antecedent social and economic variables determine labor force participation and fertility and that labor force participation affects fertility only indirectly.

Phase I entailed the secondary analysis of two previously collected data sets which report on the fertility behavior of American women of childbearing ages: (1) Growth of American Families Survey, 1960; (2) Survey of Work Experience, 1967-70.

Phase II will develop and administer an interview schedule to a sample of 400 women and sub-sample of 40 of their husbands. If these two phases of the study are completed satisfactorily, the principal investigator plans to submit a proposal entailing a national sample.

Significance to Biomedical Research and Program of the Institute: The proposal deals with a very crucial area of population well within the Institute's labor force participation and its influence on fertility behavior, but there is still much confusion about this relationship. This proposal approaches the problem in a sophisticated, fresh manner, and even if the causal model does not work, the descriptive data amassed will allow researchers to construct new conceptual and methodological approaches.

Major Findings: In the analysis of the interrelationships of work participation and fertility carried out in Phase I, it was found that early labor force experience is significantly related to family size among both most blacks and whites even with controls for marital duration or educational attainment. To the extent that early work experience measures women's work commitment this finding suggests that such commitment can influence the course of fertility. Secondly among the poorly educated women; the socio-economic status of longest held jobs both before marriage and after the formation of the family is significantly related to family size, those women with better jobs having fewer children. Job SES may be a measure either of personal work commitment or economic motives for working and therefore suggests further study on how motives may influence fertility. Finally, wife's potential earnings (as measured by educational attainment) are significantly related to family size primarily among women from low-income groups. This pattern suggests that economic motives for wife's work can influence fertility decisions. The

strong and significant relationship between the duration of work experience since the first child and family size is as suggestive of constraints on work imposed by family responsibility as it is of a reverse process. Motivation for working may reduce fertility but also, responsibilities which occur when children are born are an important factor in determining subsequent labor force participation. This secondary analysis of data suggests that women's labor force participation has complex inter-relationship with fertility.

The survey data was used to investigate the timing of the first conception and suggest that women's work preferences have little effect on this timing. Only a direct question about a "desire to postpone childbearing in order to work for awhile" was significantly related to the timing of the first conception. Those couples who expect to be more affluent appear to make less effort than other couples to postpone the first conception, but those who are in fact more affluent are likely to experience a later first conception.

The data also show that women who find outside childcare acceptable have earlier first pregnancies than those who do not. This is consistent with the idea that conflict between the mother role and the worker role is anti-natalist. An alternative interpretation is that the timing of the first birth influences later attitudes toward childrearing.

In studying the progression from parity 2 to parity 3, it appears that a preference for work may lead women to delay a third pregnancy, but that it has little influence on their eventual likelihood of experiencing it. Desired family size at marriage is quite strongly related to the parity progression ratios and while this is quite consistent with the assumption that couples make long-range fertility plans influencing their fertility behavior, it may also reflect the tendency of women to rationalize their actual fertility experience in reporting their early family size desires. In fact, the data tend to support the latter interpretation.

Proposed Course: This project, funded for a total of two years, will be completed in FY 1974.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Urbanization, Migration and Fertility in Thailand
Contractor : Brown University
Money Allocated : \$28,647 (1970); \$92,475 (1971); \$84,430 (1974)

Objectives: The purpose is to analyze census data and data from a national longitudinal survey of Thailand to determine the relationship between urbanization, migration, and fertility. The fertility data available from this national survey, cross-tabulated by residence and household type, permit their evaluation on terms of a rural-urban continuum, and their control for age differentials provide the opportunity for comparisons of fertility levels across the urban-rural continuum for differentials by religion, literacy, education, economic status, migration, and marital status.

More specifically, the study is analyzing: (1) cross-sectional and trend data on urbanization and on the relationship between fertility and socio-economic variables based on data collected in the 1960 and 1970 Thai censuses; and (2) rural and urban data from the National Longitudinal Survey of Social, Economic, and Demographic Change in Thailand collected in two rounds, 1968-1970 and 1972-1973 to ascertain: (a) total migration experience of the population; (b) the role of population and economic pressures on movement away from the village of birth; and (c) the relation between migration and fertility; (3) rural-urban labor force, occupation, and industrial differentials and data on literacy, education, and religion; and (4) data for integrating the material from the rural and urban survey operations.

Significance to Biomedical Research and Program of the Institute: The study will add to available knowledge of rural, urban and other socio-economic differentials in fertility in an area for which data have previously been non-existent, and for a culture which is quite different from that in the United States. Hence, it will add another perspective to the understanding of influences upon fertility behavior.

Major Findings: The high fertility and low mortality characterizing Thailand place its population among the fastest growing in the world. Yet within Thailand there are considerable fertility differentials by residence, education, occupation, and religion. Fertility in Bangkok was 25% below that of most of rural Thailand, corroborating the significant impact of urban residence on fertility. Education is also inversely related to fertility, regardless of residence status (urban or rural). For older women the sharper differential is between those with no schooling and those with primary schooling; for younger women achievement of more than a primary education has the greatest effect on fertility. The relationship between labor force participation and fertility in Thailand based on analysis of the 1960 census is less clear, because unpaid family workers were included in the labor force, but for the Kingdom as a whole, women engaged in farming have by far the highest fertility. There are also significant fertility differentials among religious

groups. Controlling for both age and urban-rural residence Confucian women have slightly more children than do Buddhists; Moslem women have the fewest children. Community surveys indicate that more frequent divorce and re-marriage may reduce Moslem rates but do not explain the lower Moslem rate in the youngest age group, since Moslems tend to marry early. Moslem women limit the number of their children to a greater extent than do either Buddhist or Confucian women. Greater reliance on older traditional methods of birth control or higher rates of sterility due to poorer health may be the explanatory factors.

Proposed Course: The contract, initiated in 1970, has been extended to FY 1976.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Psychological Studies of Social Norms Influencing Family Size

Contractor: University of Kansas

Money Allocated: \$83,802 (1972); \$23,895 (1973); \$59,762 (1974)

Objectives: This study will investigate the dynamics of social norms concerning large and small families--reactions to explicit and implicit social pressures in relation to normatively defined family size ideals. The following laboratory experiment is proposed: positive and negative evaluations of women as a function of the number of children (0-5) they have in relation to their age (25, 35, and 45) and career types (none, child-related career, non-child-related career, and non-child-related career with high career commitment). In the laboratory situation a sample of white, middle class, married women will evaluate women based on these variables in a design in which they will not be aware that these are the crucial variables in the experiment.

Significance to Biomedical Research Program of the Institute: The study of the dynamics of social norms concerning large and small families is highly relevant to an understanding of the determinants of fertility.

Proposed Course: This study will be extended into fiscal year 1975 because of delays in obtaining OMB clearance.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Explorations in Fertility Values
Contractor : Calepan, Inc.
Money Allocated : \$139,916 (1971) \$23,177 (1973)

Objectives: The purpose of this project is to develop and demonstrate methods to ascertain how fertility values are related to family size preferences. The principal investigator is currently developing ways in which family size preferences and the desired number of children under various economic, social, and psychological conditions can be quantified. Once the methodologies for these two tasks are refined, it will enable other investigators to measure accurately the number of children persons wish to have under various situations. Moreover, it should give insight into how people come to desire different numbers of children at different points in time.

The study entails two surveys of 300 white, married women in the 15-44 age range. For each major religious group (Protestant, Roman Catholic, and Jewish) there will be 100 women represented.

Major Findings: Dr. Terhune isolated the following values associated with family size desires: (1) economic concerns in general, providing for children in particular; (2) parental attention to each child; (3) companionship of siblings; (4) the mental strains and worries of parenthood; (5) physical work and energy requirements of parenthood; and (6) over population concerns. Furthermore, he has been able to measure relatives preferences among the family sizes. A psychometric method (expanded pair comparisons) gave useful and internally consistent results as a measure of family size preference. Analysis of the family size values show that different values are correlated with family size within Catholic, Jewish and Protestant subgroups. Religion is frequently confounded with SES but further analysis has indicated that there are religion effects, SES effects, and possibly religion by SES interaction effects. Furthermore, all the substantial correlations were negative, suggesting that it is the anticipated "costs" of having children, rather than the anticipation of rewards, which are the major determinants of desired family size. Values such as "privacy for the family members" and "freedom of parents for outside activities" appeared influential, whereas values such as "pride in children's accomplishments" and "loving and being loved by one's children" seemed to have no bearing of family size desires.

When the analysis was performed by parity level there were indeed reward incentives -- values which correlated positively and substantially with desired family size. These appeared primarily among respondents who had at most one child. Secondly, some positive correlations showed up among those who had four children. Generally, these positive correlations concerned the emotional satisfactions of having children.

Drastic differences in correlations were revealed between those who had no children and those who had one, suggesting that the arrival of the first child has a profound effect on the individual's perceptions of family size consequences. Differences among parities from one to four children were more gradual or secular, suggesting a cumulative effect of the experience of having children (as well as a filtering-out effect of those who decide to stop family growth at various points). A second study is anticipated to follow up on the research insights offered by this exploration study.

Significance to Biomedical Research and Program of the Institute: This project examines the socio-psychological factors which influence individuals' desired family size under different situations and in different points in time. This type of study has great potential in assisting family counselors in helping individuals plan their family and in assisting individuals who may be experiencing familial problems as a result of too many or too few children.

Proposed Course: This is a two-year contract. Both surveys have been completed and the data analyzed.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Family, Career and Sexuality

Contractor: Center for Policy Research
Money Allocated: \$41,946 (1972); \$14,243 (1974)

Objectives: The purpose of this research is to examine the possible relationship between ideology and fertility-related behavior. Specifically, the study includes the development of a quantitative model which will examine the relationships between: (1) family background, (2) family ideology (the attitudes and values of the respondent's mother and father, (3) the respondent's ideology, (4) the respondent's career and family role orientations, (5) sexual attitudes, (6) sexual behavior and (7) preferred family size. The model will be developed through the analysis of data already available, in 4 data sources: First, an Ideology Study of 668 students attending the University of California, Berkeley, in 1970. Second, a similar survey of Berkeley students' parents, entitled Intergenerational Study. Third, a National Student Study sponsored by the Carnegie Commission involving approximately 71,000 college students. Fourth, a National Adult Study which has data similar to that collected in the Intergenerational Study.

Significance to Biomedical Research and Program of the Institute: This project would deal with a new way of analyzing fertility behavior. The model resulting from this project should encourage other researchers to examine population dynamics from a new perspective.

Major Findings: Preliminary analysis shows that family background and father's ideology have a strong influence on the child's social ideology. For instance, the father's general liberalism or conservatism and his religious values affect his child's sexual permissiveness and not the father's specific views on sexual permissiveness. Permissiveness in turn has a major influence on attitudes toward abortion.

Proposed Course: This study was completed 31 December 1973. A final report is expected before the end of fiscal 1974.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Longitudinal Analyses of Variables Predictive of Attitudes
Regarding Fertility and Actual Fertility Patterns
Contractor : Illinois State University
Money Allocated : \$9,639

Objectives: The purpose of this study is to isolate and analyze various sexual attitudinal and demographic variables that are predictive of fertility patterns. In 1967 the principal investigator collected fertility-related data from 752 college freshmen. The principal investigator now plans to follow-up the 1967 data base with responses from these same subjects. Study subjects will be between the ages 24 and 25 at the time of follow-up. The data analysis will include sources of sex information, effect of early sexual attitudes on later sexual behavior, changes in reference groups regarding sex, and differences in attitudes and behavior concerning fertility and sex between college graduates and college drop-outs. The general hypotheses presented in this research are that fertility patterns of young adults can be to some extent predicted by knowledge of their pre-marital sexual belief.

Significance to Biomedical Research and Program of the Institute: As part of the Institute's interest in fertility behavior there is concern for the extent to which attitudes towards sexual behavior influence those factors which influence fertility behavior. This study will be an ideal opportunity to take advantage of data already collected to extend our knowledge in this area. The study is exploratory and any conclusions would have to be guarded as far as their generalized ability to a large population. However, this study will provide some unique information not before available.

Proposed Course: This is a one year contract.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Age at Marriage and Time to First Birth: A Longitudinal
Study of Parental Attitudes and Children's Personality

Contractor : College of William and Mary
Money Allocated: \$111,868

Objectives: The proposed research consists of an intensive longitudinal study of the effects of age at marriage and time to first birth on parental attitudes, children's personality development, and family interrelationships. The major focus of the research is upon first-born children within self-selected families. The sample consists of 80 two-child families, 20 in each of the four categories; early marriage and short interval to first birth, early marriage and long-interval to first birth, late marriage and short interval to first birth, and late marriage and long interval to first birth. The four groups will be matched to control for socio-economic factors and will be limited to whites. Various measures of selected attitudes and personality attributes will be made along with direct observations of family interaction several times during the three years of the project. During each visit 180 minutes of observation will be completed in 5 minute segments. The categories from Patterson's Manual for Coding Family Interactions will be used to classify the behavior observed during each segment. Data will also be obtained concerning characteristics of the parents, characteristics of the second-born children, and the interactions between parents and children within the families.

The subjects will be self-selected respondents to advertisements placed in newspapers of Williamsburg, Virginia, and surrounding metropolitan areas.

Significance to Biomedical Research and Program of the Institute: This study will expand our knowledge of the consequences of age at marriage and time to first birth for parental attitudes and the children's personalities. It is relevant to the Institute's interests in the consequences of childbearing patterns.

Proposed Course: This study is expected to extend through fiscal 1977.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Effect of Birth Order on Mother-Child Relationship

Contractor : Educational Testing Service

Money Allocated: \$88,825 (1974)

Objectives: The Principal Investigator will study the effects of birth order, sex of infant, and socio-economic status upon mother-infant interaction and upon the cognitive-intellectual and emotional development of the infant up to 2 years of age.

This study focuses on infants at each of four orders of birth; first, second, third, and fourth or higher. The sample will be selected so that each of these birth-order categories will contain 60 cases. Each category will be further divided into four (15 cases each) according to sex and socio-economic status. The subjects will be visited at ages 3 months, 1 year, and 2 years.

The independent variables recorded at the beginning of the study are sex of infant, birth order, class (middle and lower), mother's age at first birth, number of siblings, birth interval and sex of siblings. The dependent variables, 4 standardized tests (1 socio-economic and 3 cognitive) and 26 structured observations, will be recorded at the time of observation. Analysis will compare children at each of the observation periods as well as their development over the period of the study.

Significance to Biomedical Research and Program of the Institute: This study will expand our knowledge of the consequences of family size, childspacing, age of mother at birth, birth order and socio-economic status for mother-infant interaction upon the cognitive-intellectual and emotional development of the infant. Hence it is relevant to the Institute's interest in the consequences of various childbearing patterns.

Proposed Course: This study will extend through fiscal 1977.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Review of Actual and Accepted Consequences of Family Size
Contractor : Calspan Corporation
Money Allocated : \$36,900 (1973)

Objectives: The purpose of this study is to perform a critical review of the existing literature on (1) the actual consequences of family size, parents, children and general family relations, and (2) the expected consequences of family size as perceived by parents and prospective parents. The review of the consequences will give special attention to the consequences of specific family size and consequent variables. Considerations of possible confounding factors and other aspects of research designs and gaps in our knowledge will be central to the review. Special attention will be given to a comparison of "real" and expected consequences of specific family sizes. In addition to the reviews themselves, planned outlooks of the project are: (a) theoretical development of the mechanisms by which family size has certain consequences, (b) recommendations from proved research designs in substantive areas for research, and (c) implications and applications both by Government and elsewhere.

Major Findings: Preliminary findings indicate that a small but highly reliable inverse correlation exists between intelligence of offspring and family size. This appears to be a valid consequence of family size per se, because the correlation is maintained when socioeconomic status and intelligence of parents is accounted for. This finding is of major importance, for intelligence effects may explain many other correlates of family size. Since family size is generally found inversely related to social class, virtually every study that finds correlates of family size without accounting for social class is subject to question about the effects of family size per se.

While personality correlates of family size are frequently found, many of these may be due to intelligence and social class effects. Where there are controls on the latter variables, however, some personality variables do seem correlated with family size. Theory on the process by which family size can produce psychological effects is meager, as is research on the relation of family size to interactions within the family. At this time a dominant explanation of family size effects seems to lie in the diminution of interaction with parents as family size increases. This tends to diminish verbal development, which in turn has an effect on operative intelligence.

Significance of Biomedical Research and Program of the Institute: The Institute has made explicit its interest in the consequences of family size and currently there is no one source book dealing with what is already known in this area or what gaps there are in our knowledge about the consequences of family size. This research will be valuable both as a resource for others

as well as an original work. The analysis of the research designs employed by other investigators will help to guide future researchers toward the use of better designs.

Proposed Course: This project will require one year.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of Differentials and Trends in Marital Disruption, Remarriage, and the Fertility of Remarriage
Contractor : University of Wisconsin
Money Allocated : \$34,773 (FY 1970); \$42,178 (FY 1971)

Objectives: The purpose is to examine patterns of marital disruption, remarriage, and the fertility of remarriages in the United States from data obtained in the 1967 Survey of Economic Opportunity, which involved a large sample and covered the national population. The study focused on (a) differentials among socio-economic groups and racial groups, (b) patterns of divorce and widowhood in relation to age, marriage duration, color, education, age at marriage, order of marriage, sex, presence of children, place of residence, and the length of the interval between marriage and the birth of the first child, and with patterns of remarriage in relation to the above; and (c) how these patterns have changed through time. It also investigated the fertility patterns of second and subsequent marriages and how differential fertility and changes in fertility are influenced by differential marital disruption.

Major Findings: This study has found that the rate of marital disruption is twice as high for Blacks as for Whites. For black women under the age of 43, 34% of their first marriages terminated in separation or divorce while the comparable rate for white women was only 18%. The high rate of marital disruption for Blacks does not account for the large number of black households being headed by women. The lower rate of remarriage after marital disruption and the much higher incidence of widowhood is more important in explaining the large number of black families headed by women than is the incidence of marital disruption. Of those black women who have ever separated or divorced, 73% are currently (as of the survey in 1970) separated, while of the white women who were ever separated or divorced only 41% are currently separated. This is explained in part by the fact that of those Blacks who separate only 60% get divorced whereas 88% of the Whites do. This difference may be explained in part by differential access to the legal procedures for obtaining a divorce.

Early marriage is more closely associated with marital disruption than any other factor. Education and premarital pregnancy have almost no effect on the rate of marital disruption when age at marriage is controlled. The data show that if a white woman marries before age 18, the probability of her ending her marriage in separation or divorce is 50% higher than if she had waited until she was 18 or 19 to marry. For those marriages which are terminated, remarriage is extremely likely. This is especially true for men, for persons under the age of 40 or for women with fewer than two children. For men who separate or divorce before age 40, over one-third remarry within 2 years, over two-thirds remarry within 5 years and over 85% remarry by 15-19 years. Women remarry at slightly lower rates, only one-fourth within 2 years of divorce, one-half within 5 years and two-thirds within 10 years. By 15-19 years

after marital disruption 77% have remarried.

Data collected in 1970 on a national sample of women in the reproductive years (NFS) the investigator found that of those who had ever had a divorce (or separation) approximately 68% had remarried by the time of the interview. Women with no children were more likely to have remarried (72%) and those with 3 or more were least like (60%).

While rates of marital disruption are highest for conservative Protestant groups, the differences in rates according to religious groups is not extreme. A cross-sectional study of white women under the age of 45 showed that 15% had ended their first marriage in separation or divorce. Of those who listed their religious preference as Baptist or Fundamentalist Protestant, the figure was 21%. Generally speaking 14% of the other-Protestant women had ended their first marriage and 12% of the Roman Catholic women had so done. This difference is even smaller when other demographic characteristics are controlled.

The fertility implications of marital disruption and remarriage are complex. When currently married women who have experienced marital disruption are compared with women whose marriages are intact, it appears that marital disruption is associated with slightly lower fertility. Women who experience marital disruption presumably lose a period of exposure to the risk of childbearing during the period between their marriages. The effect of this lost exposure may, however, be less than might be expected because of the prevailing patterns of rapid remarriage and the possibility of intermarital pregnancy. The whole process becomes even more complicated by virtue of the fact that the inability or unwillingness to have children may increase the risk of marital disruption, while the presence of children, given marital conflict, may reduce the probability of divorce.

Significance to Biomedical Research and Program of the Institute: This study will contribute to the understanding of the effects of marital disruption and remarriage on fertility.

Proposed Course: This contract, initiated in FY 1970 terminated in FY 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Social-Psychological Correlates of Urban Fertility

Contractor: Bowling Green State University

Money Allocated: \$77,424 (1971); \$34,367 (1972); \$29,027 (1973);
\$21,658 (1974)

Objectives: This research, involving a re-interview of 754 mothers interviewed in Toledo in 1963 and a sample of 3,000 married women in the Toledo SMSA, has several interdependent objectives designed to test for possible associations, psychological and demographic variables: (1) to determine by means of longitudinal study the degree to which present family size can be predicted from alienation scores obtained from the sample of mothers in 1963; (2) to determine the extent to which the obtained differentials in fertility by alienation variables in previous research would be accentuated and refined in a community-wide sample that would include mothers who are continuing their childbearing as well as mothers who have successfully terminated their fertility; (3) to identify some of the social and demographic factors associated with differential kinds and degrees of alienation among mothers at given age, parity, and marital duration levels; (4) to determine the extent to which selected husband-wife interaction patterns are associated with differential alienation-fertility relationships; and (5) to derive a conceptual model of differential family planning and fertility behavior by combining stratification, religion, mobility, alienation, family, and information variables.

It will concentrate on attempts to understand better fertility and family planning differentials within social class and religious categories. Alienation variables will be considered as intervening between social structures and fertility, and the primary emphasis will be on specifying the conditions--of education, religion, age, etc.--under which particular patterns of alienation dimensions may be causally related to the dependent variables.

Significance to Biomedical Research and Program of the Institute: A study of the social psychological factors affecting fertility will make an important contribution to our understanding of fertility behavior, the sine qua non of the Institute's program in population research.

Major Findings: A positive association was found between high alienation scores and early marriage and high incidence of premarital and early pregnancies. High alienation scores in 1963 were associated with higher levels of fertility over the next eight years and with higher proportions of women who wanted no additional children but who had additional children nonetheless. Alienation scores remained relatively constant over the eight-year time interval. A positive relationship has been found between lower socio-economic class status and higher alienation scores.

Proposed Course: This study was funded in 1971, and extended with additional funds in 1972 because of delays in obtaining clearance. Completion of the project is expected in FY 1975.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : An Investigation of the Factors Involved in the Moral Judgment of Marital Status and Family Size

Contractor : Human Resources Research Organization, Fort Knox, Kentucky
Money Allocated: \$32,000 (1971) ; \$5,988 (1974)

Objectives: Some 500 middle class persons (100 of 16-17 year olds, 100 persons 18-20 years of age, and 300 adults divided among younger and older groups) drawn from ten organizations (PTA's, unions, alumni associations, Catholic groups, etc.) will be asked to respond to situation descriptions presenting marital status (single, married) and family sizes (0, 2, 6 children) under varied conditions of choice or choicelessness: personal desire, ability (power), and environmental circumstances. Respondents will describe their family status and how they achieved it. Various characteristics of the judges will be studied for their impact on judgments. Perceived attractiveness of the subjects judged will be examined (by bi-polar scale) to determine effects of moral judgment on this dimension. The study assumes that moral judgments are key factors in social pressures to conform, that single people and childless couples are widely viewed as blameworthy, and that this is deleterious to population control efforts. Analysis of variance will be performed to determine the effects of each of the three factors (from Heider) on moral judgment.

Significance to Biomedical Research and Program of the Institute: An understanding of population dynamics relative to future changes in the U.S. population requires a knowledge of attitudes toward family formation. This involves not only the couple but also the social context in which they live. Historically, parents, relatives, and friends have brought pressures on the couple to marry and to start childbearing early and to have large families. Thus it is highly relevant to the Institute's program to explore moral judgments as a framework for evaluating the effectiveness of programs designed to alter attitudes toward marriage or family size. The findings should be useful in counseling situations, family planning clinics, in wider educational programs dealing with marriage and family size, and in welfare programs. They should also contribute to improved maternal and infant health to the degree that they would foster changed attitudes and action programs designed to encourage the spacing of children at longer intervals.

Proposed Course: It is anticipated that this project will be completed in fiscal 1975. It has been extended several times because of delays in obtaining OMB clearance.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch

Contract Title : The Kin Network and Population Dynamics
Contractor : University of Southern California
Money Allocated : \$17,124 (1971); \$20,086 (1972); \$8,561 (1973); \$16,629(1974)

Objectives: This study will collect data on the extent and relevance of kin networks in the United States to determine how they affect fertility and migration. It is hypothesized that the greater the intensity of the kin network (frequency of contact, etc.) and of the interchange therein, the higher the fertility. The kin network may also influence the amount and direction of migration. A conference of persons experienced in the family and fertility will be convened to approve and amend the trial questionnaire and another to evaluate the results. The pretest will be conducted by National Analysts, Inc.

Significance to Biomedical Research and Program of the Institute: Research on the relationship of the kin network to fertility has high program relevance. Very little work in this area has been done.

Proposed Course: This project was initiated in FY 1971 and has been extended through FY 1975.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Effects of Purposive-Rationality, Traditionalism, and Economic Aspirations on the Process of Family Formation

Contractor : University of Kansas
Money Allocated : \$44,000 (1971)

Objectives: This study is surveying 1,000 first-time parents classified on the basis of the extent to which such factors as goal orientation, traditionalism, and economic aspirations affected their decision as to the number and spacing of births.

Significance to Biomedical Research and Program of the Institute: This study of how persons make decisions concerning family formation is definitely relevant to the Institute's program. The "training of respondents to use a self-administered questionnaire facilitates follow up over time and imparts to this study a methodological as well as substantive importance, since one of the problems with longitudinal studies is their very great expense. If this methodology works, it can serve as a model for other longitudinal studies at more reasonable cost."

The project should make contributions to the sociology of the family and be particularly helpful in understanding how and why persons decide to have different numbers of children. As a result, it should prove useful for persons in the area of family planning.

Major Findings During Year: Of the variables tested, economic aspirations and dimensions of traditionalism tend to be related to fertility intentions and desires as expected. However, on the criterion of instrumentalism (the belief that one can influence one's future and not simply be subjected to fate), it was found in preliminary analysis that wives with the highest instrumental orientation want and expect slightly more children rather than the fewest, but the more instrumental wives do favor a greater number of years for spacing between children. This finding may reflect a Middle Western approach to family size consistent with certain other studies in which people said they wanted 3 or more children but were still concerned with environmental questions.

Proposed Course: This project, initiated in 1972, is expected to be completed in FY 1975.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Work and Childcare Patterns of Women After the
Birth of Their First Child

Contractor : University of Kansas

Money Allocated : \$32,369 (1973)

Objectives: The purpose is to expand an ongoing study to obtain data regarding the rationale for women working or not working outside the home and characteristics of their work patterns since the birth of their first child. Socio-economic background, attitudinal and aspirational variables will be analyzed for their effect upon the extent to which women work after the birth of their first child. Special attention will be given to how the kind and availability of childcare facilities or arrangements effect the mother's work activity.

The data will be obtained in a second questionnaire to be administered in a longitudinal study in three Kansas counties of the process of family formation among 1,000 young couples who had their first child in 1971. Each couple completed a first questionnaire in 1972 under an NICHD contract designed to find how sequential decision-making as to the number and spacing of births was affected by three independent variables: purposive rationality or goal orientation, traditionalism, and economic aspirations.

Significance to Biomedical Research and Program of the Institute: This study builds on one already underway and should provide information about the factors that encourage or discourage labor force participation of a woman when her first child is about 2 years old. The subject is of particular cogency at this time when significantly large numbers of women born in the postwar period are entering the prime labor force, marriage, and family-formation age group, many of whom are reported in the labor force. For example, the proportion of mothers of children under six years of age working outside the home doubled from 15.7% in 1952 to 30.4% in 1969--or nearly as much as for mothers of children 6-17 years of age.

Proposed Course: This research is scheduled to run for two years. OMB approval of the questionnaire submitted October 1973 is still pending in April 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Reimbursable

Agreement Title: Relationship between Age at Marriage, Birth Intervals and Total Fertility

Contractor : U.S. Bureau of the Census (reimbursable agreement)

Money Allocated: \$140,000 (1971); \$165,000 (1972); \$75,000 (1973)
\$75,000 (1974)

Objectives: This project has enabled the Census Bureau to collect, tabulate, and analyze data on fertility from the Current Population Surveys of June, 1971, 1972, and 1973. The agreement permitted the addition of questions on children ever born, childspacing, birth order, birth expectations, and marital history to the June 1971 Current Population Survey. In June 1972 and 1973, the project permitted the addition of questions on children ever born, birth expectations, and date of last live birth. The general objectives of the project are to provide data for the analysis and interpretation of current fertility trends and to develop better methods of projecting fertility rates into the future.

Major Findings: The most notable finding of this project has been the decline in the number of births expected by married women aged 18-24. In 1973 as in 1972, the average number of births expected by such women was only 2.3, compared with 2.9 in 1967 and 3.1 in 1965. The proportion of young married women expecting a total of two children increased from 37 percent in 1967 to 56-57 percent in 1972 and 1973. While the proportion expecting three or more children declined from 56 percent to about 30 percent in 1972 and 1973. The number of children expected by young women is now consistent with the replacement level of fertility--that is just enough births to replace the number of people in the parent generation. If such low rates continue indefinitely, the population of the United States will stop increasing, except for immigration, in about 65 years.

Significance to Biomedical Research and Program of the Institute: This study will make an important contribution to the analysis of trends in fertility, by analyzing age at marriage and timing of births within marriage in relation to levels of fertility. It will also provide basic information for an understanding of fertility behavior and its changes in the United States over time. The project thus bears directly upon a primary concern of the Institute's program.

Proposed Course: NICHD continued supporting the Census Bureau Current Population Survey through the June 1973 Survey as a vehicle to obtain data on fertility, childspacing, and birth expectations and to provide overlap with the National Survey of Family Growth undertaken by the National Center for Health Statistics. Advance data were published in Current Population Reports: Population Characteristics (P-20, No. 254, Oct. 1973) and final data will be published before the end of June 1974. The Bureau of the Census is underwriting the inclusion of these fertility questions in the June 1974 CPS.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Reimbursable

Agreement Title: Childspacing and Current Fertility: 1970 Census

Contractor : U.S. Bureau of the Census (Reimbursable Agreement)

Money Allocated: \$350,000-\$400,000 (3 fiscal years, 1973-1975)
\$5,000 (1973); about \$220,000 net (1974).

Objectives: From the 1970 census a special magnetic tape file will be derived dealing with women aged 14-54, including information where applicable on husbands, children, and other household or family characteristics. The project will provide data by socio-economic characteristics for several population groups on: (1) age at marriage, intervals between marriage and first birth, and birth intervals for later births by cohort to determine any changes in childspacing patterns from cohort to cohort; (2) the effect on fertility of separation and of marital dissolution and remarriage; (3) unintended childbearing (pre- or immediately post-marriage or after age 35 following a long interval without bearing children; and (4) social and work roles of women. Data on current fertility, as measured by births and birthrates, 1960-69, will replace less precise 1960 census data on children under five years of age. A brief report on major results will be published early, followed by a special 1970 census Volume II report of some 400 pages on Childspacing and Current Fertility, and the magnetic tape file will be made available for other researchers to use.

Significance to Biomedical Research and Program of the Institute: The potential fruitfulness of this project for the study of fertility correlates is inestimable. The report will provide basic data on childspacing of the U.S. population, and the data file will permit detailed fertility analyses heretofore impossible to make, facilitating the study, for example, of the consequences of alternative childbearing and childspacing patterns. A further major value of this research lies in its usefulness in relating fertility process to such factors as employment, occupation, income, housing conditions, etc.

Proposed Course: This research is scheduled for a period of two years, covering parts of three fiscal years, 1973-1975 and likely to total \$350,000-\$400,000.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Methods and Techniques for Using the 1960-1970 Census
One Percent Public Use Samples
Contractor : National Data Use and Access Laboratories, Inc.
Money Allocated : \$235,403 (1972) ; \$243,035 (1974)

Objectives: The purpose of this proposal is to develop techniques to facilitate the wider use of the 1970 Census Public Use Samples (PUS). The project emphasis is on the handling of very large files by writing programs using COBOL and FORTRAN program to merge different types of records, create smaller work tapes, and provide programs for cross-tabulations and analysis of data. The proposal also includes the writing of users' manuals and provision of classroom instruction in the use of these programs which will be available in the public domain at low cost.

Major Findings: The principal investigator has completed work on the PUSH software system, which will enable researchers in the field to use commonly available statistical methods (such as the Statistical Package for the Social Sciences, SPSS) with the Public Use Sample. This is a major advance in making the Public Use Sample tapes available to a large number of people. The tape and manual have been distributed to approximately 32 sites and the manual has been distributed to several hundred researchers. The manual was published through GPO and the tape distributed by DUALabs. The principal investigator has also developed a CENTS-AID program to allow investigators to use the public use sample with the very fast cross-tabulation program (CENTS) developed by the Census Bureau. The CENTS-AID manual is being published by GPO and will be distributed in the same manner as the PUSH manual. DUALabs is also offering seminars and instruction in the use of both PUSH and CENTS-AID.

Significance to Biomedical Research and Program of the Institute: The U.S. Census is an important source of data for demographers and other social scientists, as are other censuses which are made available from time to time. The sheer volume of the 1970 output, however, requires special effort. The needs of most researchers could be satisfied with select variables and records on a single manageable reel tape but the problem is to produce that reel or tape for individuals. DUALabs is a unique facility, incorporating previous experience with census, and several years work in this field.

Proposed Course: This project is scheduled to terminate in April 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Determinants of Fertility, Migration, and Population
Change of Counties in the United States, 1950 to 1960

Contractor : University of Georgia
Money Allocated: \$81,781 (1971)

Objectives: This study will analyze the trends and patterns of population change for the total white and non-white population of all counties in the United States from 1950 to 1960. Changes in this period will be explained in terms of fertility, mortality, and net migration, the major emphasis being on fertility and migration and upon the white and non-white differentials. The study also investigates the socio-economic and demographic determinants of each of the three components of population change among both white and non-whites. County fertility rates and differentials, for example, will be examined by age (and age standardized), sex, color composition, and by levels of urbanization, industrialization, income, occupation, education, and related factors. Finally, tabulation and organization of the data by color, not previously compiled on a uniform basis will provide benchmark data for all counties in the U.S. for the 1950-1960 period for comparison with similar trends in the 1960-1970 decade. Plans have already been made for future computation of the 1960-1970 components of county population change by color. Maps will be prepared showing county birth, death, and net migration rates, and natural increase rates, by color. Birth rates of counties which gained and lost population will be systematically examined, and constellations of counties with similar fertility and net migration patterns will be identified.

Major Findings: The principal findings are that whites in predominantly Spanish-American counties and nonwhites in American Indian counties had the highest crude birth and total fertility rates of whites and nonwhites, respectively, in the five major types of counties. The traditionally high fertility of the Appalachian Region had disappeared by 1950-1960, for both the white and non-white residents of this area had the lowest crude birth and total fertility rates of the five major ethnic or cultural groups. Regression analyses revealed that, other than dominant cultural group, the age structure of counties was the most important explanatory variable in accounting for total, white, and nonwhite crude birth and total fertility rates.

Significance to Biomedical Research and Program of the Institute: This efficient utilization of massive data already processed and coupled with further data from the censuses and the county and city data books, for the 1950s is a useful approach to the analysis of population change in clusters of counties

and regions of the United States, and is basic to a projected analysis of the dynamics of the 1960s. This study is exceedingly relevant to the study of population processes in the United States and, therefore, to the Institute's program, both from a descriptive and analytical point of view.

Proposed Course: This project was completed during fiscal 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Study of Family Formation and Fertility - Key Trends
and Patterns
Contractor: University of California
Money Allocated: \$171,088 (1973)

Objective: The purpose of this study is to develop a data base to facilitate the study of the relationship between illegitimacy and social changes in the family and sexual behavior, including abortions and venereal disease. The investigator will continue work on data linkage of marriage records and birth certificates to determine legitimacy status of mothers delivering children in 1967, other birth records and divorce records, to develop comparative data on patterns of marriage, divorce and separation of a sample of married and unmarried mothers in 1967. In addition, investigator will develop a questionnaire for a sample of California households to study the process of family formation and fertility. And he will do a comparative study of trends in illegitimate and legitimate fertility in California 1966-1971, including abortion and reported V.D. cases and do a comparative analysis for approximately 20 other cooperating states having varying abortion statutes, marital patterns, and V.D. experience.

Significance to Biomedical Research and Program of the Institute: This study will contribute to knowledge concerning illegitimacy, abortion, and V.D. in relation to sexual and family formation patterns and prepare the groundwork for a more extensive study of how social patterns relating to sex and marriage affect the incidence of illegitimacy, abortion and the current rise in V.D. rates. It therefore has immediate relevance to both health and population-related behavior.

Proposed Course: This contract was continued in FY 1974 and will be extended with additional funds in FY 1975.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : New Estimation Techniques for Demographic Analysis
Contractor : University of North Carolina
Money Allocated : \$75,579 (1970); \$172,876 (1971); \$136,628 (1974)

Objectives: The purpose is to develop sensitive indicators of change in fertility patterns, i.e., appropriate measures for the analysis of the lengths of "closed" intervals (between births) and "open" intervals (since the most recent birth). Such data are assuming increasing prominence as predictors of natality change and indicators of the effects of family planning programs. The contract will develop new measures, assess their accuracy, reliability, and sensitivity, and compare various procedures for collecting the necessary data. To evaluate the measures, an existing microsimulation model will be modified and other special purpose models utilized as necessary. The project should contribute to more general methods analyzing current natality data and to the choice of appropriate indices of change.

Experiments using augmented simulation models are being devised to (a) simulate reproductive performance, postulating different behavior patterns reflecting different levels and rates of population change, (b) simulate different schemes of sampling in data collection in real populations, (c) calculate from such simulated data the values of the measures derived above, and (d) assess the behavior of these measures in response to the conditions of the experiments. The project has been broadened to include: (a) a comparative study of the sensitivity of alternative indices to changes in fertility, emphasizing indices based on birth intervals as compared with the more traditional measures of total fertility and age-specific fertility rates, and (b) development of documentation of the POPREP simulation model of the general biological model of population growth and the associated computer program SURVEY used for simulation of sample surveys.

Major Findings: Efforts to date have focused on modifications of existing microsimulation models of fertility behavior to use in carrying out the purposes of this study. Various experiments with these models have dealt with a number of aspects of fertility measurement, among them measures of "fecundability," or the monthly probability of conception, which generally varies between .15 and .20 among women not using contraception.

Significance to Biomedical Research and Program of the Institute: The development of more sensitive indicators of current changes in fertility has important implications for both the collection of vital statistics and the evaluation of the effectiveness of public health programs, including those relating to family planning.

Proposed Course: The contract, initiated in FY 1970, will continue into FY 1976.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of Physicians' Attitudes Toward Abortion
Contractor : Temple University
Money Allocated : \$208,053 (1971); \$44,000 (1973)

Objectives: The major purpose of this study is to assess physicians' current perceptions, understandings, and attitudes toward birth control methods which may be considered as possible abortifacients. The doctors' attitudes concerning criminal as well as legal (therapeutic) abortions and relevant general attitudes toward fertility intervention and birth control will also be examined. Some 1,500 interviews will be given to a national probability sample of M.D.'s and osteopaths whose practice makes the subject of fertility control a reasonable occurrence. The interview schedule will be developed by video and audio taping a series of "In-group" discussions with physicians from four regions of the United States: the Northeast, South, Midwest, and West. The tapes will be analyzed and questions concerning how best to approach the subject of birth control and abortion will be determined. The questionnaire will include the following areas: definition of pregnancy and of abortion; perception of peers and of the public mood toward abortion; individual perceptions of patients and their circumstances toward abortion; etiology in the correlates of attitudes toward abortion and specific objections to abortion; and the actual behavior of the physician regarding prescriptions of birth control methods and when appropriate, the performance of abortion.

Significance to Biomedical Research and Program of the Institute: Since there is a trend toward the liberalization of abortion statutes and the physician is becoming increasingly involved, it is crucial to determine how the medical profession views abortion. This study accordingly has a great deal of relevance to the Institute's program.

Proposed Course: This project, initiated in 1971, was originally funded for 2 years and will be extended through FY 1975.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Final Household Survey to Complete the Family Planning Evaluation Project

Contractor : University of North Carolina (Chapel Hill)
Money Allocated: \$204,615 (1973); \$76,341 (1974)

Objectives: The primary purpose of this project is to complete a large-scale controlled-action experiment of an area sample survey of 16 American cities involving a total sample size of about 3000 ever-married women in order to evaluate the effect of family planning programs on the fertility and fertility behavior of communities. This post-survey was made in each city in 1969. Data from the two area sample surveys of ever-married women living in low-income census tracts will be integrated with other data from monthly interviews of newly-delivered mothers, quarterly vital statistics tabulations, operating statistics of the projects, and other data resources (all paid for by other grants) for final interpretation of the impact of family planning programs and the reproductive behavior of the communities they serve. Effects are being measured on four levels: knowledge and attitudes toward family planning, use of services, contraceptive behavior, and reproductive events.

Significance to Biomedical Research and Program of the Institute: Although this project has operational research aspects not described here, it basically examines the premises on which family planning projects operate, and their consequences for the behavior of communities. The initial survey was funded by a grant from Maternal and Child Health Services (MCHS) of the Department of Health Education and Welfare. This project takes an evaluative look at the effect of family planning programs on the fertility of the areas in which the programs are located. It concentrates therefore on demographic impact rather than upon the health of mothers attending, which was the focus of the MCHS grant, or the efficiency of service delivery, which is the usual criteria for family planning programs. It is thus highly critical to the understanding of factors which affect levels of the birth rate.

Proposed Course: This contract is funded for two years, from December 1972 through November 1974.

NICHD ANNUAL REPORT

July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : On the Consequences of Reproduction: A Utility Model
of Reproductive Behavior

Contractor : University of North Carolina

Money Allocated : \$455,648 (1974)

Objectives: The purpose of this study is to test a utility model of fertility designed to predict the probability of various reproductive events to couples. The basic premise is that individuals regulate their reproductive behavior in light of their expectations and perceptions of the consequences of reproduction for them. Variables will include perceived consequences in terms of costs and rewards of facilitating or preventing a subsequent birth. The study will also be concerned with the congruence between expected and actual consequences and the effects of the congruence or lack thereof on subsequent expectations. Completed family size is viewed as resulting from constantly changing fertility behavior in the face of changing life circumstances and the changing perception of the consequences of reproduction. It takes into account changes relating to the anticipation of a child, the birth of a child, the impact that this has on future childbearing, etc. Each of these events affect the perception of subsequent anticipated costs and benefits--the utility functions--and alter behavior accordingly.

A sample of 3,000 women living in selected low income areas in 16 U.S. cities interviewed previously under an existing NICHD contract will be asked various questions concerning the perceived assets and liabilities of adding children to their families. A sub-sample of 1,200-1,500 women under 30 years of age, married and husband present, will constitute a group to be re-interviewed at least twice. In addition, 500 women and their husbands will be interviewed in suburban areas in the cities.

Significance to Biomedical Research and Program of the Institute: Testing of the important hypothesis that fertility behavior is "rational" within the perceptual framework of the actors--low income and middle income alike--will, if verified, have important implications for policy at a variety of levels.

Proposed Course: This research is scheduled to run for 3 years.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Development of Methodology for Evaluating the Demographic Impact of Organized Family Planning Programs on the United States

Contractor : Center for Family Planning Program Development, New York
Money Allocated: \$112,302 (FY 1973)

Objectives: The major purpose of this project is to estimate the effects on fertility of the provision of subsidized family planning services. Subsidiary purposes are to identify the factors accounting for geographic differences in fertility and the factors associated with varying levels of family planning program input. The dependent variables will be various measures of fertility based on 1970 Census data on reported numbers of children ever born and numbers present in households. These fertility indicators will be computed for groups classified by age, color, and poverty status for single counties or groups of counties. The major independent variable is the level of family planning services provided (reported patient loads averaged for fiscal year 1968 and calendar year 1969 divided by an estimate of the population of low income women in need of contraception). In addition, there will be several intervening variables: proportions of women in intact marriages, average number of years of school completed, female labor force participation at ages 15-44, percent urban, and estimate of the 1965 general fertility rate.

Significance to Biomedical Research and Program of the Institute: This is the only national study of the impact of family planning service levels on the fertility of the low-income population. It should provide answers to questions that people have raised about the effects of voluntary family planning programs. As such, it will have direct relevance to the formulation of policies regarding population and family planning in the United States.

Proposed Course: The contract, initiated in FY 73, is expected to be completed in FY 75.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Determinants of Fertility Change in Tamil Nadu, India

Contractor : University of North Carolina

Money Allocated: \$180,000 (1972)

Objectives: This study aims to investigate whether there is a threshold of social and economic development after which family planning action programs will make more rapid progress. The approach proposed is "observational" rather than experimental. The existing geographic differences in social and economic development and in family planning action program inputs in one state (Tamil Nadu) in India will be used to select an appropriate sample of areal units (Community Development Blocks and Hamlets), and the fertility and family planning behavior of the populations in these units would be studied.

The total project has two parts. In Part 1, the available data for all 375 Community Development Blocks in Tamil Nadu would be compiled and analyzed to assess the variation in fertility or contraceptive acceptance attributable to: (a) differences in family planning program inputs, (b) differences in socio-economic level of development. These data would also be used in selecting a sample of Blocks for Part 2. Within each of the 54 sample Blocks 12 Hamlets (of 1,000 population each) would be selected. All the sample units would be drawn so as to represent all combinations of program inputs and levels of socio-economic development to permit repeating the analysis in Part 2 with more accurate data on program outputs.

Significance to Biomedical Research and Program of the Institute: This study affords an excellent opportunity to investigate whether there is a threshold of social and economic development above which family planning action programs may make more rapid progress:

Proposed Course: This proposed research, initiated in 1972, has been scheduled to run three years.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of Social Mobility and Fertility Control
Contractor: University of Colorado
Money Allocated: \$116,263 (1972)

Objective: The purpose of this study is to test an hypothesis concerning the urban mobility process and its relation to the utilization of family planning practices. The investigator hypothesizes that as residents of central city slums in the Philippines improve their earning capacity they move to squatter settlements on the periphery of the city and in the process both the economic value of children decline and their aspirations for their children increase, with the result that the squatter settlement residents develop desires to limit their family size and adopt family planning. He is testing the hypothesis in Davao City through demographic surveys of central city slums and squatter settlements and intensive interviews of a sample of households in those areas and examination of family planning records. Ethnographic interviews with key informants in each area studied will be conducted for the history of the settlement. The study is expected to contribute substantial reinforcement to the mobility theory in respect to adoption of small family values and birth control practices. The investigators are familiar with the area in which the study is being conducted.

Significance to Biomedical Research and Program of the Institute: This study will contribute substantially to knowledge regarding factors influencing reduction of fertility generally and in an Asian setting particularly, and thus contribute to the improvement of theory of fertility. It will also contribute to the improvement of family planning program policy in suggesting strategic locations for program efforts in underdeveloped countries.

Proposed Course: The study is scheduled to run for two years and will be completed in FY 1974 or early FY 1975.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Social and Psychological Factors Affecting Fertility,
Family Planning and Clinic Utilization

Contractor : Wake Forest University
Money Allocated: \$95,936 (1972)

Objectives: The purpose is to re-analyze in-depth data collected in three KAP-type interview surveys concerned with black males and females in Winston-Salem, North Carolina, and Hackensack, New Jersey, and with Spanish females in Costa Rica. Emphasis will be placed on psychological and social-psychological variables as they relate to fertility, family planning and clinic utilization. For women with a high risk of high fertility various social-psychological measures will be devised and related to different sets of explanatory variables. Twelve different analyses will be undertaken to test out hypotheses.

Six analyses will pertain to black women in the United States regarding: (1) definition and indexing of the context of "risk" (biological, societal, and medical); (2) early sexual experience and attitude toward sex; (3) comparison of male- and female-headed black households as to differences with respect to such factors as education, parity, number of current sex partners, and attitude toward abortion; (4) poverty as a "culture of poverty" vs. the relationship to intervening variables, such as education, age at first marriage, legitimacy of first conception, and age first learned to prevent conception; (5) religion, social class and abortion; and (6) consequences of being born out of wedlock.

The six analyses pertaining to Costa Rican women will be concerned with such aspects as: (1) modernization and modernism; (2) correlates of fertility; (3) trends in childspacing; (4) credibility of sources of family planning; (5) urban-rural differences in fetal and infant mortality; and (6) comparison of consensual and legal marriages.

Significance to Biomedical Research and Program of the Institute: This project is relevant to the NICHD program relating to the interface between population and family planning in that it goes beyond the clinical approaches taken by such governmental agencies as AID, and the National Center for Family Planning Services. It also brings in a cross-cultural aspect, which relatively few studies have done.

Major Findings: The studies undertaken among blacks in New Jersey and North Carolina communities and among women in Costa Rica indicate the following: (1) blacks have accepted the national family size ideal but evidence a wide-range of acceptable number of children around that ideal point; (2) in Costa Rica, education, age at first conception, nature of emotional relationship with father of first child and age when woman learned of contraception were

determining factors in a woman's life situation; (3) consensual unions, contrary to the literature, do not depress fertility; (4) women in consensual unions differ from those in legal unions primarily in terms of social class and related KAP (Knowledge, Attitude and Practice) factors; (5) psychological factors may be of greater importance in determining family planning clinic attendance than previously reported in the literature, i.e., fears and untoward experiences at a family planning clinic and with contraceptives are more important than a woman's demographic characteristics in predicting whether or not she will continue attending a clinic or drop out; and (6) the most important factor in communication about family planning in Costa Rica is the positive or negative reinforcement of friends, relatives and neighbors rather than the quality of the information transmitted. Findings have been disseminated through professional journals. In addition, authorities responsible for family planning both in the United States and in Costa Rica have used these findings in planning their objectives and programs.

Proposed Course: This contract, initiated June 1972, is expected to be completed early in FY 1975.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : An Analysis of the Causes and Patterns of Rural to
Urban Migration of the Poor

Contractor : University of California (Berkeley)

Money Allocated: \$17,684 (1972)

Objectives: This study investigated the causes and decision-making process underlying the migration behavior of the rural poor and explored the potential for various public policies which could influence migration from rural and small areas to big city ghettos. It tested a series of alternative causal models for explaining migration while investigating in depth a model of individual adjustment to economic and social displacement. The principal investigator will conduct a secondary analysis of data previously collected. Data for 1800 lower socio-economic respondents have been collected in 1969 by ABT Associates and funded by OFO, including information on perceptions, experiences, and attitudes as well as migration behavior. Approximately half of the study population were migrants from rural to urban areas, the others were current residents of rural areas. Three migrant streams were involved: Southeastern Blacks, Appalachian Whites and Southwestern Spanish Americans.

Significance to Biomedical Research and Program of the Institute: The study population is important in terms of explaining the causes of migration patterns of the poor. Economic and other pressures which may force poor families to migrate to urban areas have implications for the planning and implementation of public health programs.

Major Findings: Most migrants interviewed had increased their income and viewed urban opportunities more favorably than rural conditions and opportunities, yet one-third desired to return to a rural area. Most poor migrants had considered only one destination--and that only a short time before migrating, had moved only where friends or relatives already resided, and had a job lead.

One-fourth of rural interviewees were considering migration, but the preference was clearly for remaining in-state, even at some cost in income. In the absence of rural jobs, the rural interviewees might advise young people to migrate but did not perceive that the migrants would like the city. Most non-migrants tended to be older, married, property owners, participants in government assistance programs, and to have more children and dependents.

The study explored the phenomenon that many of the rural poor may be "trapped" by their poverty or may value their membership in a rural social community more than the potential improvement to be gained through migration. Rural

interviewees most critical of rural opportunities and most praiseworthy of urban opportunities did have the least education, income and employment prospects, and more family responsibilities and other ties (or binds) to the rural area.

The relationship between migration perceptions and experiences was generally similar for the three migration streams studied. Appalachians, however, tended to migrate out of economic necessity, prefer the rural lifestyle, and considered returning to the rural area. "Pull" factors and concern for amenities were more dominant for Blacks, and discrimination problems were more salient to Southeast Blacks than to Southwest Spanish-Americans.

Methodologically, the study tested an overall model of migration using multiple regression with dichotomous dependent variables to portray migration as a choice of locations based on maximizing the access to opportunities. Perceptions of opportunities are shown to be more powerful predictors of migration than the demographic, sociological and other factors measured.

Policy recommendations designed to alter or redirect the urban migration of the rural poor include: strategies for penetrating the informal information networks used by the poor; facilitation of commuting or migration to regional growth centers as effective alternative to migration to large central cities; and the need to improve opportunities for employment and services in the rural area:

Proposed Course: The contract, initiated in FY 1972, was completed in FY 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Rates and Consequences of Population Change: The Growth
and Decline of Counties in the Mountain Region

Contractor : Colorado State University (Fort Collins)
Money Allocated: \$85,480 (1971)

Objectives: This study described the population changes that have occurred in the Mountain Region (8 states, 279 counties stretching from Montana to Arizona) during the period 1950-1970. Almost one-half of these counties lost population between 1950 and 1960 and more than one-half declined in population during the decade 1960-1970. The main interest centers on the consequences of population change--consequences to public finances, retail activity, housing utilization, and agricultural activity.

Censuses of population, government, businesses, housing and agriculture, and other published and unpublished pieces of information (available from state and local sources) were used. Components of change as well as indices of social welfare, such as doctors per capita, use of health services or school retardation, and grade continuation rates were explored to clarify some of the relationships and problems investigated.

Significance to Biomedical Research and Program of the Institute: Depopulation is one of the more important, yet neglected, aspects of the population problem in the United States. Most of this nation's counties either lost population or experienced net out-migration in the last two decades, and very large geographic areas are coming to have sparse populations. Few studies have investigated the process or the consequences of such population losses.

Proposed Course: This study, funded in 1971, was completed in FY 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Review of Research Findings on Rural-Urban Migration
with Annotated Bibliography

Contractor : TRACOR, Inc.
Money Allocated: \$30,818 (1972)

Objectives: This project, originally funded by OEO, was continued by NICHD when OEO decided to terminate support of projects on migration.

The purpose is to prepare a summary of research findings on rural-urban migration, an extensive annotated bibliography by author, and a subject matter index.

Significance to Biomedical Research and Program of the Institute: The development of annotated bibliographies such as this are of tremendous benefit to established investigators, students entering this field of research, and to policymakers who want an overview of what has been done in this field.

Proposed Course: The Price-Sikes draft of "Rural-Urban Migration in the United States: An Annotated Bibliography and Synthesis" has been edited by staff and is undergoing technical editing for publication by the Government Printing Office as a "CPR" (Center for Population Research) monograph in FY 1975.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Analysis of Migratory Responses to Employment Opportunities

Contractor: Battelle (Columbus, Ohio) Laboratories

Money Allocated: \$53,500 (1972)

Objectives: The aim of this research is to clarify the relationship between migration and certain demographic and economic variables, particularly potential employment. For a sample of 100 counties, drawn from a 14-state area, in-migration rates for 1955-1960 can be obtained from a special tabulation of the 1960 census. Rates of net-migration by area, sex, and color, will be obtained from the Bowles-Tarver series and, after adjusting for a five-year period (instead of the decade over which they were computed), rates of out-migration will be obtained by subtracting rates of in-migration from adjusted rates of net-migration. With age, sex and education controlled, a clearer relationship between migration and economic opportunity should emerge. Migration rates for each group will be regressed on variables measuring aggregate employment opportunities and group specific employment opportunities in county labor market areas. The 1955-1960 data will be used to predict 1965-1970 migration and will be checked against cross-tabulated data from the 1/100 sample for 1960 and 1970.

Significance to Biomedical Research and Program of the Institute: The proposed research has high program relevance, fitting into a mosaic of our existing contract migration studies relating to: (1) the analysis of the Social Security file covering 90% of employment in the United States to obtain data on inter-regional, metropolitan, and non-metropolitan migration patterns by sex, color and age, (2) migration from Appalachia, and (3) the impact of depopulation upon economic and social institutions in the Rocky Mountain area. The present research proposal relating migration and economic opportunities should make a real contribution to the understanding of the determinants of migration, a subject of increasing policy interest.

Major Findings: (1) The hypothesis that migration is determined in part by the existence of job opportunities is tentatively proved; (2) education and age have a combined relationship upon migration--education makes one more mobile and the relationship becomes stronger and clearer with increasing level of education. Individuals with 16 or more years of education have high average rates of in-migration and responsiveness to employment opportunities, since they are likely to be aware of alternative job opportunities in various areas, have been relocated by their companies, or have contracted for employment in a new community upon completing college. High average in-migration rates for females in the 20-24 age group are probably due to education and marriage effects. For females with 13-15 years of education, the additional education may have opened new job opportunities or resulted in marrying a more

educated and mobile spouse. Males over 40 years of age do migrate, but primarily in response to specific job opportunities rather to generalized opportunities.

Proposed Course: This project was completed in FY 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Reimbursable Agreement Title: Income and Migration in the United States
Contractor: U.S. Bureau of the Census
Money Allocated: \$190,000 total
\$60,000 (FY 1974)

Objectives: The purpose of this reimbursable agreement is to study how the absolute level of individual and family income and type of income interact with other variables to influence decisions to move, the distance moved and region of destination. It will also study such consequences of migration as effects on earnings, trends in income and fertility, and progress of children in school. To meet these objectives, the 1960 census data relating to migration will be retabulated to be compatible with tabulations available for the 1970 census and the Current Population Survey 1961-1970.

Significance to Biomedical Research and Program of the Institute: Migration is the demographic process by which individuals and groups adjust to their social and physical environment. The evaluation of the degree to which this is successful, and the factors which influence the process is especially relevant to the program of the Center for Population Research, which has as one of its emphases the study of consequences of population growth and change. It will provide information of concern to the Public Health Service and DHEW in that it will document the role of poverty status and welfare assistance programs in determining decisions to move or stay, and the economic and educational consequences of such moves for individuals, particularly those in poverty status.

Proposed Course: This reimbursable agreement was initiated in 1974 and will be completed in either FY 1975 or 1976.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : An Analysis of the Spacing of Pregnancies, Births, and Completed Fertility in Relation to Socio-economic Status, Intergenerational Mobility and Aspirations for Children, Aberdeen, Scotland

Contractor : Brown University

Money Allocated: \$48,469 (1971)

Objectives: The study investigated: (1) how spacing of pregnancy varies with the type of terminations of previous pregnancies, the stage in the family life cycle, age at marriage, age at first pregnancy, and socio-economic status; (2) how the spacing of live births, the number of children ever born, and the length of fertility span vary by age, age at marriage, age at first pregnancy socio-economic status, social mobility, and mobility aspirations for children; (3) the life conditions under which women are likely to resort to terminations and sterilizations; and (4) how the size of the family of origin, and position on a traditionalism scale are related to social mobility, present socio-economic status, aspiration for children and fertility. The data are based on a probability sample of 2,000 resident women of Aberdeen, Scotland, who had at least one live birth during the period October 1950-September 1955.

Major Findings: Confirming the frequently expressed view that high aspirations for oneself and one's children are an important determinant of low fertility, Aberdeen, Scotland data demonstrate that social mobility influences low fertility. Couples who are moving from a medium status to a high status position exhibit behavior similar to that of people who have always been in a high status position. The fertility of couples whose families have been in a high status position is the lowest and the couples who have moved into such a position exhibit lower fertility than those in a medium status position though not so low as for those who have held high status for some time.

Significance to Biomedical Research and Program of the Institute: The Aberdeen data provide a rich source of information on how the outcome of one pregnancy influences subsequent pregnancies, how family origin variables relate to child-spacing, and how fertility related to social mobility.

Proposed Course: This study is scheduled to be completed in FY 1974.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Distribution and Differentiation of Population
Within Metropolitan Areas
Contractor : University of Michigan
Money Allocated : \$110,936 (1971)

Objectives: This study analyzes 1970 U.S. census tape data for 25 SMSAs and compares these with census materials for four Canadian metropolitan areas to determine: (1) the degree of residential segregation of different socio-economic groups and of families in different stages of the life cycle; (2) the pattern of segregated housing of different types and ages; (3) the relation of housing type to socio-economic characteristics of occupants; and (4) variations in population distribution in different metropolitan areas.

Significance to Biomedical Research and Program of the Institute: The distribution and differentiation of population in cities is of major importance, but there have been few empirically based studies on this topic. This study will provide a framework for considering the similarities and dissimilarities of a sufficient number of metropolitan areas in the U.S. and Canada to give meaningful results as to population dynamics and will develop generalized analytical tools for the study of other cities. This research goes beyond a study of segregation patterns to look at associated factors. It is thus highly relevant to NICHD goals and should be useful to urban planners as well.

Major Findings: Census data was analyzed to describe migration trends up to 1970, especially those patterns involving blacks between 1960 and 1970. This showed that blacks continued to migrate away from the South to the three other regions although the volume and rate of out-migration were lower in the 1960s than in either previous decade. While the South, Northeast and North Central regions have traditionally lost white population and the West gained, this pattern no longer holds. The Northern regions continue to experience a net out-migration of whites, but the South is now gaining whites.

There is no evidence of a substantial in-migration of Northern-born blacks to the South nor do the figures reveal a substantial return migration of blacks from the North or West to the South.

During the 1960s, the out-migration from rural areas and the concentration of both races within metropolises continued. Within many metropolitan areas, there is a racial difference in the location of population: blacks are frequently much more concentrated within central cities than are whites.

Analyzing migration data for large central cities disclose that during the 1960s, as in the 1950s, there was a substantial out-migration of whites. In such places as Detroit, Cleveland and St. Louis, net out-migration equalled one-third of the population present at the beginning of the decade. In most cities the volume of black in-migration was lower in the 1960s than in the

previous decade. Several large cities - including Cleveland, New Orleans and St. Louis - experienced a net out-migration of blacks.

In the 1960s, as in the 1950s, blacks who migrated into metropolitan areas were higher in socioeconomic status than non-migrants and two components of the migration stream can still be identified. There are relatively high socio-economic status inter-metropolitan migrants and lower status in-migrants from non-metropolitan areas. In most locations, the volume of black in-migration declined and the mix of the two components has shifted since migrants from rural backgrounds are now less numerous.

Proposed Course: This project, begun in FY 1971, is expected to be completed in FY 1974.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Demography of the Black Population

Contractor : University of Texas

Money Allocated : \$87,792 (1973)

Objectives: The purpose of this study is to describe and analyze trends in the demographic characteristics of the black population, and compare these with trends in the white population. The basic analytical approach will be a cohort analysis by sex and color. The principal investigator did a related study for the 1960 census, Changing Characteristics of the Negro Population: A 1960 Census Monograph, U.S.G.P.O., 1969. Part of this project will be to check the projections outlined in the earlier work, seek to explain changes from 1960 and 1970, and to update the earlier monograph. The principal investigator will focus on how changes in social policies, e.g., the Civil Rights Act of 1964, may have influenced changes. Projections will be made for future trends. Although the period from 1960 to 1970 will be emphasized, the 1930, 1940, and 1950 censuses will also be utilized. Topics to be dealt with are: income, education, home ownership, place of residence by region by region, urban and rural, relationship to head of household, employment status, and the beginning of a series on region of residence by state of birth, migration, population distribution, occupational structure and change, and marital patterns and household composition.

Significance to Biomedical Research and Program of the Institute: The dynamics of the black population are not well understood and, until relatively recently have not been systematically analyzed for their own value. Rather, the demographic changes of the black population were considered in a secondary manner, as something to compare the white population with. This project will take the opposite tack and analyze the black population, considering the white population only as a reference point for differential change. This project will consolidate what is known about the dynamics of the black population. There is sizeable literature on blacks and a number of reports on the demographic aspects of the blacks. They usually concentrate, however, on a particular subject and/or region, e.g., black migration to the North and their levels of income. This report would take a holistic approach to the black population.

Proposed Course: Expected to be completed in FY 1975.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of Optimum Population Levels

Contractor : University of Virginia
Money Allocated: \$58,275 (1972); \$95,000 (1973)

Objectives: This study is exploring different approaches to, and a methodology allowing the calculation of, an "optimum level of population" in terms of increases or decreases in an index of quality of life (IQL). The major effort is toward a modification of the Gross National Product (GNP) to adjust for the amount of the national income accounts which are directed to the dis-economies produced by population growth. The study will attempt to project changes in the so-called IQL as population increases and as population distribution changes under the assumption of various technologies relating to mineral consumption, water use, energy, and other natural resources.

Significance to Biomedical Research and Program of the Institute: This is one of the very few studies in the Behavioral Sciences program of the Center which addresses itself to the problem of measuring the consequences of population growth and change. Much of the current concern over the effects of increasing population size has been based on data and on assumptions which have not always met with general acceptance. This study is attempting to develop a methodology which will make the consequences of population growth and change much more specific and identifiable, at least in economic terms, and therefore should be of great benefit to policy makers in the determination of what population goals should be pursued.

Proposed Course: This study was funded for one year in fiscal 1972 and was renewed for an additional year in 1973. Additional funding is anticipated if progress under the current contract justifies it.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Economic and Environmental Consequences of Population Change

Contractor: Resources for the Future
Money Allocated: \$283,126 (1974)

Objectives: This study will attempt to estimate the major effects on the economic, the resource base and the environment of a number of alternative demographic developments, both nationally and regionally, that may occur during the next 30-50 years. Three models will be used and they will be supplemented by special studies where necessary.

The first model will operate on a national level and is an extension and modification of the model used in the investigator's contribution to the Population Commission Report (Vol. III). Using various demographic, economic and technological assumptions as exogenous variables, this model provides output in the form of labor force by economic sector, economic output by 185 sectors, investment by economic sectors and so forth. The model also predicts resource requirements (both by primary minerals and energy) by economic sector and pollution emission and treatment cost by economic sector.

The second model uses outputs from the first model and disaggregates effects to the regional level. The economic, resource and environmental effects of alternative patterns of geographic location and economic activities will be studied in more than 200 regions. A regional breakdown is important for studying environmental problems since damages from pollution emission very depending on the region being studied.

The third model will use the outputs from the first two models and focus on the agricultural sector, investigating land and water requirements and output pollutants from pesticides, chemical fertilizers, etc. Study at this level is important because the agricultural sector is a principal user of land and water and contributes to pollutants with a long half-life.

A series of scenarios will be developed pertaining to likely changes in factors (taste, technology, etc.), which could influence the relationship between population and the economic and environmental variable. For each scenario the model will be run with alternative assumptions about demographic developments. By comparing the results for a scenario when different assumptions about population are made, the influence of the demographic variables can be ascertained. When the same demographic assumptions are used in the reviewing of different scenarios the investigator can ascertain the relative importance of population vis-a-vis other factors.

The results of the 3 models will be compared with other studies for plausibility. Finally an attempt will be made to draw conclusions about topics not specifically included in the model. Using the national model, for example, the contractor can investigate whether a movement towards a stationary population would make it more or less difficult to maintain full employment.

Significance to Biomedical Research and Program of the Institute: This project is relevant to the Institute's goal of understanding the consequences of population size and distribution. A central issue in the study of population growth rates is how they affect general welfare of the population. This project will elaborate the effects of population size on general welfare and especially on the economic and resource bases. These effects most directly influence the quality of life experienced by the population. The regional component of this project will provide valuable data on the effects of pollution, agricultural practices and land or water use patterns.

Proposed Course: This is an 18 month study.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Population Growth and Employment Growth in Metropolitan Areas

Contractor : The Urban Institute (Washington, D.C.)
Money Allocated: \$177,206 (1974)

Objectives: The purpose is to design a model quantifying the interaction of demographic change and employment change as they have occurred in the recent past in some 311 SMSA and smaller metropolitan areas covering over 70 percent of the U.S. population.

The principal base for this research is a file already assembled with respect to population and employment by industry and economic and social characteristics for these metropolitan areas for 1965 and 1970 on the basis of unpublished tapes of County Business Patterns for 480 activities representing a 4-digit partitioning of the Standard Industrial Classification supplemented by data from other sources and estimates of the number of employees in particular industries.

Significance to Biomedical Research and Program of the Institute: The focus of this study on the interrelationship between economic growth and population distribution provides a new dimension to internal migration--one of the research areas in the Institute's program. Knowledge of the economic basis of the distribution of population is important in assessing the availability and private-public mix of the delivery of health services.

Proposed Course: The contract should be completed and a report of findings submitted toward the end of FY 1975.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Consequences of Metropolitan Population Stability or Decline

Contractor : Scientific Analysis Corporation
Money Allocated: \$41,356 (FY 1974)

Objectives: The purpose of this study is to address theoretical and policy issues related to the consequences of metropolitan population stability or decline based upon empirical investigations of 30 selected American metropolitan areas which have experienced periods of population loss since 1940. It will compare two samples of cities over the period 1940-1970: one which has grown continuously and one which has had episodes of non-growth. A number of questions will be studied in this comparison, not so much to prove or refute specific hypotheses as to identify the different kinds of effects that accompany non-growth. The study will also involve intensive study of a selected sub-sample of cities with stable or declining populations. This project will explore the policy implications of population distribution as it refers to allocation of federal spending between areas and strategies for preventing localized deterioration of welfare under non-growth.

Significance to Biomedical Research and Program of the Institute: The focus of this project is on the consequences of distribution of population, a topic clearly relevant to the aims of the Institute. The general problem of distribution is whether it contributes to the maximum adjustment of people to their social and physical environment. A central issue in the argument for population growth vis-a-vis stability or decline is the ultimate effect of these processes on general welfare. In examining the economic consequences, as well as effects on general welfare, of population decline or stability in selected cities in comparison with other selected cities whose populations have grown, this project promises to contribute new insights into the effects of population distribution.

Proposed Course: This is a one year project.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Population Redistribution and Depopulation in Non-Metropolitan Pennsylvania: Social and Economic Correlates and Policy Implications

Contractor : The Pennsylvania State University
Money Allocated: \$294,056 (1972) \$2,913 (1974): \$49,765 (1974)

Objectives: This is a complex undertaking which will investigate in detail, historically and contemporaneously, the non-metropolitan population changes in the State of Pennsylvania. That State is a large, well-established area with a long history and highly diversified non-metropolitan sector. The investigators propose to create a data bank of relevant historical-statistical information and to create a typology for the non-metropolitan areas in order to analyze the most important processes shaping change in those areas. Data will be derived primarily from the census but several other sources will be used and field studies will be conducted. The studies will focus on transport, technology, changing labor force roles of females, attitudes toward metropolitan living, and others - which have shaped the non-metropolitan sectors of the population.

Significance to Biomedical Research and Program of the Institute: This proposal will differ from earlier research on related topics in demography and related fields by its strong focus on a great range of non-metropolitan characteristics and trends in a highly advanced region. The issue of population dynamics in non-metropolitan areas affecting population distribution has been a very neglected area for research. Several proposals are currently pending in Congress and elsewhere for increasing the attractiveness of non-metropolitan areas. Whether or not these are well advised depends upon the understanding of the forces and processes affecting such areas and upon projected distributions of population and economic opportunities.

Proposed Course: This multi-faceted research program will be completed in FY 1975.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Support of Publication of Population Index

Contractor : Princeton University

Money Allocated: \$98,288 (1971); \$72,984 (1973); \$90,879 (1974)

Objectives: The purposes of this support are to permit the continuation of the publication of Population Index as a quarterly journal containing bibliographic information and special articles in the population field; to computerize the preparation of final copy for photo-off-set reproduction of the Index; and to develop a computerized retrieval service for the production of special bibliographies.

Significance to Biomedical Research and Program of the Institute: The need for knowledge about changes in population size and composition and the way in which these are related to socio-economic factors is well recognized by policy makers in the field of public health. For these and other users of demographic resources, an accurate and comprehensive guide is essential. NICHD funding permits the maintenance of a bibliographic tool of tested utility and the broadening of its usefulness for searching and finding. The Index over the past 36 years has also carried a broad range of articles on current developments in demography.

Major Findings: In an effort to conform to government policy, Population Index has achieved self-support with respect to publication costs. Continued support is being sought only for the salaries of those engaged in bibliographical work in the field of demography. With the financial support thus far provided and the acceptance of NICHD guidelines, the Index staff has (1) increased office efficiency, (2) improved the quality of the publication, e.g., by the inclusion of a "Checklist of Current Government Serial Publications Containing Vital or Migration Statistics" and the addresses of sources consulted, and (3) made the Index available reasonably close to its issue date. Computerization of the Author Index beginning with the April 1973 issue has permitted off-set reproduction to save typing costs; the Geographic Index was computerized some years ago. Increasing circulation (up 6% between October 1972 and October 1973 issues to about 4,550) and the inclusion of advertising (14 advertisements in the first year) have also helped to offset rising costs of producing and distributing the Index.

Proposed Course: This contract, initially funded in 1971, will be considered for a one-year renewal beginning in September 1974.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Processes of Demographic Development in Imperial
Russia and the Soviet Union
Contractor : The Ohio State University
Money Allocated : \$52,700 (1970); \$107,155 (1971)

Objectives: The purpose is to study the mutual relationship between population growth, economic and social change in Imperial Russia and the Soviet Union. In order to identify the long-term factors that determine and influence demographic changes, the study will explore the determinants of Soviet population growth from a long-run point of view, which also indicates the early stages of change during the Imperial Russian period, and to appraise the Soviet efforts to design and implement population policies to influence fertility patterns and other demographic variables. The principal themes of the proposed research will be those concerned with the implications of the developmental process for population growth, with major attention given to the question of fertility.

Significance to Biomedical Research and Program of the Institute: The study of demographic development during a process of economic development and social change during the 18th, 19th, and 20th centuries in a society which shows both European and Asiatic influences is of the highest relevance in understanding the process of population change in the world at large. The findings will increase knowledge of the factors associated with declining fertility, the use of contraceptives and of abortion, and will provide some insight into the effectiveness of a variety of population policies.

Major Findings: A general decline in fertility over much of the Soviet Union has become evident since the early 1960s. Net Reproduction Rates--less than unity in all but Central Asia, the Caucasus and scattered parts of the RSFSR--imply ultimately declining growth of the total population rather than the long-run moderate rates of increase that most Soviet demographers and planners regard as desirable. Changes have been dramatic, and regional differences, very great. In European Russia the downward trend in fertility was persistent. Fertility declined from 1900 to 1959 in both urban and rural areas, with the rural level being about 25% above the urban. It declined sharply in the rural areas of European Russia, in the 1930s, which were also affected by exceedingly high out-migration as a result of agricultural collectivization and industrialization. However, Soviet Central Asia and the Caucasus, except for the Georgian and Uzbek Soviet Socialist Republics, evidenced little or no decline in fertility. Implications of this research are: (1) the recently declining fertility will result in a decline in the population of working ages beginning in the 1980s; (2) the crisis in labor allocation and distri-

bution will be aggravated if present regional fertility differentials are maintained and if certain national groups in areas of relatively high fertility in Central Asia/Caucasus continue to be unresponsive to economic or other incentives to move; and (3) Soviet demographers are becoming increasingly concerned that non-Slavic groups through their higher fertility may overwhelm the Slavic groups in the long run.

Proposed Course: The original contract, initiated in 1970, was renewed with funding for FY 1971 and FY 1972. A no-cost extension was given in FY 1974 to complete the project.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Patterns of Emotions and Feelings in Sex-Related Behaviors
Contractor: Vanderbilt University
Money Allocated: \$64,625

Objectives: The purpose is to test hypotheses concerning the emotional and attitudinal components of experiences in relationships involving love, sex, contraception and abortion. The underlying theory holds that the emotion system is a principal motivational system, with the emotions possessing unique properties in interacting drives (hunger, thirst, pain, avoidance, etc.) in sexual matters. Thus, other emotions interacting with the sexual urge can affect sexual behavior in a variety of ways, and may be extremely important in the effective practice of contraception. For example, a man who holds a woman in contempt may be less likely to use adequate contraceptive methods even though pregnancy may be unwanted. The research will be conducted among (a) 1,000 college students at Vanderbilt University and Middle State Tennessee University; and (b) 150 each unmarried pregnant women (at a Florence Crittenton home) and 150 married women pregnant for the first time.

Significance to Biomedical Research and Program of the Institute: The interaction between emotions, sexual and contraceptive behavior may be a crucial factor in explaining unplanned and unwanted pregnancies. A woman's pregnancy and childbearing may result from feelings of insecurity and inadequacy or by guilt in having sex for pleasure or may represent an effort to fight off the emotional distress of loneliness or rejection. With more adequate information on how discrete emotions influence the sexual urge, sexual behavior, and reproduction, more effective methods can be developed for re-enforcing or changing attitudes consonant with the well-being of the individual family and of the broader society.

Proposed Course: This research is scheduled to run for 2 years. Approval requested of OMB June 1973 still awaiting action as of April 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Correlates of Family Size and Childspacing in the United States and Puerto Rico

Contractor : Boston College

Money Allocated: \$150,612 (1972); \$25,308 (1974)

Objectives: The purpose of this study of 500 families of high school students in middle class suburbs of Boston is to show the effects of family size and the spacing of children in the "quality of life" of the parents and of the children. Variables on the parental side include: age, socio-economic status, religiosity, education, work history, childbearing attitudes, recreational patterns, financial conditions, membership in voluntary organizations, degree of integration into the community, and rationale for birth of each child. Variables on the children's side include: academic achievement, intellectual capacity, vocational and educational aspirations, personality, social relations, work history and expected age at marriage, family planning attitudes and subsequent life style. Interviews and questionnaires in addition to peer ratings and school records of the children were used. This project replicates the key aspects of another NICHD project conducted in a middle class suburb of San Juan, Puerto Rico, by the same co-investigators. New variables included child-spacing and the impact of family size on the lives of parents.

Findings: Even in the relatively prosperous (median family income above \$20,000) Boston suburbs studied, more children of small families than of large families (73% vs. 54%) perceive themselves as being of "above average intelligence"--a fact which will be checked against test scores. A higher proportion of small--family than of large-family housewives (53% vs. 30%) are now working.

Significance to Biomedical Research and Program of the Institute: The analysis of how family size and birth spacing affects the welfare of both parents and children is a major focus of the program of the Center for Population Research, NICHD.

Proposed Course: This contract is scheduled for completion in FY 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Role and Fertility Patterns of Urban Mothers
Contractor: Columbia University
Money Allocated: \$88,828 (1972); \$9,687 (1973); \$59,394 (1974)

Objectives: This project is exploring the relationship between female roles and fertility. The major hypothesis of the study is that timing of the first birth greatly influences the number and timing of subsequent births, the manner in which a woman views herself in relationship to familial roles (i.e., mother and wife), and how she perceives herself in non-familial roles, (e.g., student and worker). The principal investigator is conducting an exploratory study of women in the New York area between the ages of 15 and 29 who have recently born their first child. The research instrument concentrates on the determinants and timing of the first birth. Specific areas to be explored are the socialization process the subjects underwent in terms of their family of origin, friends, social economic status, religion, race, and their perception of their mother and father. This project is innovative in the sense that most studies which deal with fertility and, especially those that try to project future fertility patterns, have concentrated on the most recent birth which may or may not be the first birth. It is also a methodological study which will develop a research strategy and instrument which, if successful, will be utilized in a larger study.

Major Findings: Preliminary analysis of this data shows that the median age of first birth for this sample was 21.9 years and that 27 percent of these births were illegitimate. Approximately half of the women (aged 15-29) had used contraception from the time sexual activity was initiated, and only 12 percent had never used it. This study showed that a young woman's failure to use contraception while saying that she was not trying to become pregnant was often the result of weak motivation or poor contraceptive information. Accessibility of contraceptives and interpersonal barriers were also factors. Most (62%) of the women who said their first birth was unplanned said they wished it had come later or never, while only 4 percent of the women who had planned their births felt this way.

Significance to Biomedical Research and Program of the Institute: This project has great potential for increasing our knowledge of the factors which determine fertility at the individual's level. As a result, the information which may ultimately come from the project will be valuable for the medical and allied professions in advising persons how to better plan their families.

Proposed Course: This project was initiated on March 15, 1972 and extended to June 30, 1973. During the contract period, the research instruments and findings of the pilot study will be evaluated to determine if further research in this area will be fruitful.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of the Growth of Mexican-American Families

Contractor : University of California at Los Angeles

Money Allocated : \$71,811 (1971); \$32,768 (1973); \$23,176 (1974)

Objectives: The objective of this research is to design and carry out an initial and exploratory analysis of the determinants of fertility and family planning among Mexican-Americans in Los Angeles County. Next to Blacks, Mexican-Americans constitute the most important minority in the United States and have the highest fertility of any major cultural or ethnic minority. The study emphasizes the effects of social structure and social norms on fertility and family planning but also considers socio-psychological variables. A multistage probability sample of 1200 Spanish-surname women of childbearing ages is being interviewed to collect the following data: (1) reproductive experience, including a history of births and pregnancies as well as number of children expected or desired; (2) past and anticipated contraceptive usage; and (3) determinants of both reproductive and contraceptive experience. More specifically, these determinants include knowledge of, and attitudes about, contraceptive practices and family size, extent of orientation toward or involvement in Mexican-American society, socio-economic status and social mobility, family structure, characteristics of community residence, migration history and religious practices. Various multi-variate techniques will be used to analyze the data.

Significance to Biomedical Research and Program of the Institute: The Mexican-American minority is of particular interest because it comprises one of the minority groups and enclaves of high fertility in a nation with generally declining fertility.

Major Findings: The principal investigator and the Survey Research Center at UCLA have found that they must take into account the wishes and concerns of Mexican-American women and organizations in designing the sample, using women interviewers suggested by their organizations, etc. This points up the increasing sensitivities of respondent groups in undertaking social science research.

Proposed Course: This contract funded in June 1971, will be completed in late FY 1974 or in FY 1975.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Fertility Dynamics of Cuban Refugees

Contractor : University of Miami

Money Allocated : \$176,584

Objectives: The purpose is to investigate the relationship between fertility, migration, cultural and socio-economic changes among a population of 300,000 Cuban refugees--or half of the U.S. total--living in the Miami-Dade County area of Florida. The study will test a number of hypotheses relating fertility to minority status, religiosity, migration and acculturation within an overall theoretical framework of "convergence theory," namely, that fertility as well as social and other values and behavioral patterns of the Cuban refugee population will converge over time with those of the general population. A combination of questionnaires administered to 800 women, family histories obtained from in-depth interviews of members of a sub-sample of 25 households and ancillary data from the Cuban Refugee Office and the Research Institute for Cuba and the Caribbean will be used to compare fertility (children ever born to married women 20-44) in Cuba and the United States and changes during the period of adjustment to a new environment.

Significance to Biomedical Research and Program of the Institute: In an effort to understand the fertility behavior of the U.S. population, considerable interest has been focused on the Spanish-American population. The Cuban refugee population represents an important segment of this population. While sharing with other segments the Spanish-American culture, it also exhibits striking differences with respect to socio-economic status and conditions under which migration has occurred. Research on this group complements several other NICHD-supported studies on Spanish-American populations in the United States, all of which emphasize the search for influences on fertility behavior.

Proposed Course: This research is scheduled to run for two years. An appeal to an OMB disapproval pending as of February 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Research on the Consequences of Childbearing and Child-spacing Patterns

Contractor : American Institute for Research

Money Allocated: \$66,680 (1973)

Objectives: The Principal Investigator will conduct a secondary analysis of the project TALENT longitudinal data bank at the American Institutes for Research. The data was collected at four different times. In 1960 a national sample of over 400,000 high school students were given two-day battery of tests and questionnaires dealing with information on the student's personalities, abilities, interests, aspirations, and background (e.g., age of father when respondent was born, sibship position of the respondent, number of brothers and sisters). Follow-up interviews and questionnaires, one, five and eleven years later, yielded information on schooling, career choice, marriage, timing of the birth of the first child, and subsequent family development.

These data allow the examination of three kinds of relationships: (1) The childbearing patterns of the students' parents will be examined for their consequences to the students' personalities, abilities, interests, and aspirations. (2) Relationships between the characteristics in 1960 and subsequent schooling, career, marriage and childbearing patterns of the students, (3) Consequences of childbearing and child-spacing patterns of TALENT marriage partners and career aspirations, socioeconomic statuses, etc.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in both the antecedents and the consequences of marriage and childbearing patterns.

Proposed Course: This study will extend into fiscal 1975.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch

The Contraceptive Development Branch continues its mission to develop an array of effective, safe and reversible methods of contraception for both men and women. The wide range of studies, from clinical trials of drugs and devices, chemical modifications of existing known drugs, to fundamental research in the reproductive systems in both sexes, has been supported through 128 contracts, some of which are short-term and others long-term studies.

Two important trends have been noted in the course of the past year. One has been an increased interest in the development of new contraceptive measures for men. The other has been development of new drugs and drug formulations which have, or are about to reach the stage of clinical evaluation and long-term toxicity studies. Accordingly, the commitment of funds to the two broad areas of endeavors listed below, which has been more or less equal in the past, has begun to shift into the product development category.

A. Directed Fundamental (Biomedical) Research

Basic studies in female and male reproductive biology are essential to the development of new insight into the details of the reproductive process.

Studies in the Female

Detection of Ovulation

The knowledge of the precise time of ovulation is of paramount importance from both an academic and practical point of view. Fertility regulating methods that do not rely on mechanical or chemical agents are employed by a relatively large segment of our population. Thus, we need to know the time of ovulation if we are to develop indirect, predictive indices which can be helpful in determining the infertile or "safe period" of the cycle. Methodology has been developed for the detection of the cumulus mass (which contains the ovum) within the ampulla of the fallopian tube.¹ This event takes place only several minutes after ovulation so that for practical purposes it can be directly associated with ovulation. Transducers which pulse light through the oviduct, measure the change in optical transmission when the cumulus mass passes through the light beam. Application of similar methodology to the monkey is complicated by the fact that the oviduct in this animal has much thicker walls than the rabbit and consequently the signal is weaker. However, placement of the optical transducer closer to the fimbria (ovarian end of the oviduct) has resulted in better noise to signal ratio. Further research is under way to improve both the transducer and the associated surgical techniques. Experiments in chronically-instrumented monkeys are scheduled for the coming year.

An indirect method for the prediction of ovulation,² based on chemical changes in vaginal fluids, is presently being investigated. The method is based on the hypothesis that high estrogen levels, characteristic of the preovulatory

phase of the menstrual cycle, produce a specific pattern of vaginal metabolism. The research program is aimed at ascertaining the levels of volatile fatty acids in the vagina during the preovulatory phase and whether or not the pattern is reproducible. Preliminary results from normally cycling women volunteers suggest that a useful pattern may develop. Many more cycles will have to be studied to prove or disprove the validity of the method.

The Ovum and the Oviduct

The analysis of the contractility of the rabbit and monkey oviducts are beginning to shed light on the characteristic patterns that are observed in each species.³⁻⁵ The overall level of contractile activity is higher in the rabbit than in the monkey.³ After ovulation the pattern of oviductal activity in the rabbit is very regular, with a highly characteristic power spectrum. This type of contractility pattern favors pickup and propulsion of the ovum through the oviduct.⁵

Continuous 24-hour recordings of oviductal motility are being obtained from a totally implantable two-channel telemeter in unrestrained monkeys.⁴ Data from 31 monkey-months of transmitted contractile recordings, representing 17 ovarian cycles of various experimental design, have been collected on both hard copy and magnetic tape. These data are being processed through a specially designed computer program which provides the information in tabular, histogram and graphic form and correlated with the physiological status of the animals. Thus, for the rabbit there are data relating contractility to the rate of ovum transport; such data are not available for the primates.

Further experiments on fluid transport in the oviduct support earlier evidence of considerable species difference.³ The ciliary current in oviducts of macaques and humans is consistently toward the uterine end, whereas in the Rabbit and sow it is directed towards the ovarian end of the duct. The transport of isthmic contents to the site of fertilization is very rapid in the pig and the rabbit whereas it is much slower in the monkey and the human.

Study of the chemical and physical properties of the oviductal fluid and its formation has been hampered because of a lack of sensitive means for measuring the secretions. Accurate and sensitive micro- and ultramicro-analytical methods have now been developed for fluids obtained from the rabbit,⁶ as well as from rats and mice.⁷ Through the use of these techniques, it is now possible to determine the properties, rate of formation, and influence of hormones and drugs on oviductal fluid.

Studies of the secretory process of the oviduct have continued. The incorporation of ³⁵S-sulfate into oviductal mucin and its subsequent microscopic visualization shows that in the precoital stage, localization is in the Golgi components of the endoplasmic reticulum whereas 6 to 24 hours postcoitally the localization is in the secretory granules of the isthmus and ampulla of the duct.⁸

Significant progress has been made in the studies of RNA synthesis in the fertilized ovum, during the early stages of development in the rabbit. Of interest is the finding that ribosomal RNA is synthesized as early as the 16-cell stage

of development and a burst of ribosome production occurs in the early blastocyst. If maintained in vitro, blastocysts which have gone through a burst of ribosome production become blocked or arrested, and ribosomes are not released to the cytoplasm.⁹ This may provide a novel lead to fertility control if a means of preventing the "burst" of ribosomal formation could be developed which would thereby arrest the further development of the blastocyst.

The Cervix

Very little is known of the physiology and biochemistry of the process whereby changes in the peripheral levels of steroid hormones will induce profound changes in the nature of cervical secretory activity. In the past year, several studies were initiated which entail attempts to demonstrate receptor-sites for the female hormones,¹⁰⁻¹² the chemical nature of various components of the cervical mucus,^{13,14} the dynamics of sperm transport through the cervix^{15,16} and the possibility of developing antisera to certain cervix-specific molecules.¹⁷ Little actual data can be reported at this time.

The Pituitary - Ovary - Corpus Luteum System

Interference with corpus luteum (CL) function is one of the pragmatic approaches to the development of new contraceptive agents since the CL is the major source of hormonal support for the very early stages of zygote development. The interference can be produced either by directly blocking certain synthetic pathways within the CL¹⁸ which are necessary for progesterone production or by blocking the release of gonadotropins which support the CL.¹⁹ A number of compounds have been identified which inhibit certain luteal enzymes in vitro. Their activity in vivo is presently being explored in the pseudopregnant rabbit model system. One of the active compounds that has been identified is aminoglutethimide and its potential utility as a contraceptive agent has been assessed. In its present form the compound exhibits toxicity, and research is in progress to separate the two components (isomers) with the hope that the biologically-active one will have considerably lower toxicity. Studies dealing with triamcinolone (a corticosteroid), and thyrotropin releasing hormone (TRH) as blockers of ovulation have continued. Evaluation of these drugs in baboons has been complicated by the stress of handling, daily blood drawing or anesthesia. These stressful procedures have over-ridden the ovulation-blocking effects of the drug. Preliminary reports by others on clinical trials show that TRH does not block ovulation; the implications of the baboon studies must be cautiously interpreted.

To further promote our understanding of the pituitary-ovarian system there has been continued activity in the field of gonadotropin purification. Human chorionic gonadotropin (hCG) has been purified in large quantities and separated into its constituent subunits.²⁰ Similarly, sheep luteinizing hormone (LH) has been separated into its subunits and these materials together with hCG are being made available to the scientific community.²¹ A further activity in this area involves the purification of monkey urinary FSH.²² The aim of this project is the development of a preparation which is sufficiently pure to be utilized as an antigen for radioimmunoassay purposes in these highly important experimental animals.

The proper understanding of ovarian-pituitary interrelationships is dependent, to a very large degree, on the availability of analytical methods for measuring the circulating levels of various reproductive hormones. Progress has been accomplished in the development of an automated immunoradiometric assay machine.²³ Development of this device will permit the continuous sampling and automatic measurement of various pituitary hormones. It will help in assessing the nature of hormonal interactions on a very rapid basis.

Prostaglandins

The investigation of the effects of prostaglandins (PGs) on a variety of reproductive endpoints has continued. Administration of $\text{PGF}_{2\alpha}$ ² to women in the follicular phase has had an equivocal effect.²⁴ In some of the subjects ovulation has been delayed whereas in others there was no noticeable effect. Similar administration of $\text{PGF}_{2\alpha}$ to cycling rhesus monkeys has been without demonstrable effect on cycle length. It has been conclusively demonstrated that bovine endometria are very rich in arachidonic acid which is a precursor of PGs.²⁵ Direct administration of arachidonic acid into bovine corpora lutea is followed by a depression of progesterone output. Injection of $\text{PGF}_{2\alpha}$ into sheep follicles causes the regression of the adjacent corpus luteum.²⁶ The presence of receptors for PGs in the endometrium and myometrium of the human has been confirmed. Binding of $\text{PGF}_{2\alpha}$ to the rabbit oviduct has also been demonstrated.²⁷ As an extension of the studies on PG-binding to various uterine components, an investigation was conducted in which the influence of PGs on calcium (CA) binding within the uterine muscle was studied.²⁸ $\text{PGF}_{2\alpha}$ and -E_2 decreased ATP-dependent Ca-binding and also promoted the release of Ca from uterine muscle components. It is believed that the effect of PGs on Ca is directly related to their stimulatory action on muscle.

Studies of Male Reproduction Processes

The penetration of the ovum by the spermatozoon is thought to involve the action of proteolytic enzymes. Several of these enzymes are located in the anterior region of the sperm head, the acrosome.²⁹ The best understood acrosomal enzyme has been termed acrosin, and its function is to facilitate the passage of the spermatozoon through the outer membrane of the ovum, the zona pellucida. Inhibition of this enzyme by either naturally-occurring or synthetic inhibitors blocks fertilization.³⁰ Methods are being devised for the large scale isolation of acrosin from human and rabbit spermatozoa.^{30,31} Another acrosomal enzyme involved in the fertilization process is the corona-penetrating enzyme. Its assumed function is to facilitate the passage of sperm through the cells surrounding the ovum. Further research on the isolation and purification of this enzyme is required before its function is fully understood and its inhibition can be explored for fertility regulation.

The study of sperm enzymes and the role they play in sperm-ovum interaction has been paralleled by a search for inhibitors of these enzymes from natural and synthetic sources. It has been observed that the seminal plasma contains a variety of inhibitors that can block fertilization. Two inhibitors of acrosin have been found in human semen,³¹ and several "decapacitation" factors have been isolated from the seminal plasma of the bull and the rabbit.^{30,32} Decapacitation is as yet a poorly understood process by which spermatozoa lose

their capacity to fertilize ova. Reversibility of the process is easily accomplished by reincubation of spermatozoa in the uterus. Two peptides with decapacitation activity have been identified in bull seminal plasma and their amino acid composition established.³⁰ Under normal condition, they are part of a larger protein molecule and only appear as peptides after enzymic digestion. The future goal of this research is the identification of the structure of these peptides, their synthesis, and testing as antifertility agents.

The epididymis continues to be the subject of intensive investigation. Recent data have revealed that the testosterone content of bull sperm is considerably greater than that of cauda epididymal plasma, and hence, sperm entering the epididymis may be fully saturated with male hormone.³³ The presence of two androgen-binding proteins have been demonstrated in the epididymis of adult rabbits. One of these binding proteins is produced in the testes and is confined to the caput epithelium. The other binding protein is present in all segments of the epididymis.³⁴ In some recent studies, epididymal responses were examined in adult male rats 30 days following castration--a time when sperm and steroid binding protein have disappeared. In these experiments, testosterone and dihydrotestosterone provided only partial maintenance of the epididymis.³⁵ Thus, it may be that both testicular and epididymal binding proteins are necessary to maintain the full integrity and function of the epididymis. Since androgens are known to maintain spermatogenesis and the production of androgen-binding protein in the hypophysectomized animals,³⁶ it will be important to ascertain whether or not, under the circumstances, epididymal function is fully maintained.

The preceding biochemical studies are further substantiated by morphological examination of epididymal tissues from rats with tubular ligations. Ligation of ductuli efferentes is followed by a decrease in height of the epithelium and an even more striking decrease in overall diameter of the initial segment of the ductus epididymis. Electron microscopy shows a persistence of those cellular activities and organelles associated with absorptive function of the epididymis, but there is a marked depression of the sparsely granulated endoplasmic reticulum. The results suggest that the initial segment is highly dependent on androgens coming via androgen bound protein.³⁷ Studies designed to provide basic information needed to elucidate mechanisms of action of potential antispermatogenic agents are proceeding in a number of laboratories. Several areas have been identified as possible points of interference: i) The nuclear protamines undergo oxidation of free sulphhydryl groups to form intermolecular disulfide bridges thus becoming a complex keratinoid network stabilizing the genome. The initial phase of disulfide formation is proposed to occur during nuclear condensation of the spermatid, with progressive formation of additional disulfide bonds induced during epididymal maturation of the sperm.³⁸ ii) Microtubules of Sertoli cells have been positively identified and evidence obtained of their role in maintaining the integrity of the seminiferous epithelium. Experiments with Colcemid have identified these microtubules as the target for induced reversible sterility.³⁹ iii) Evidence of the regulatory function of the Sertoli cells suggest these cells are important targets to interference.⁴⁰ Recent data indicate that these cells may be the source of the androgen-binding proteins.³⁶ iv) Examination of Sertoli cells from a variety of pathological states suggests that they are not as injury-resistant as has been previously postulated. The accumulation of watery vacuoles seems to be a nonspecific response of Sertoli cells to a variety of insults.³⁷

Understanding the meiotic process in the testes is of central importance to the area of reproductive physiology, as it is an absolute requirement for the formation and differentiation of the haploid gamete. In the past, studies of testicular function have concentrated mainly on morphological changes occurring in the testes and on the steroid secretion from the testes. A direct analysis of the meiotic process during gametogenesis in the male had not been achieved. Therefore, research has been undertaken to develop methods of isolation, separation, and characterization of meiotic germ cells in rat testes. By using testes from particular aged animals, it has been possible to obtain cell fractions containing 68 to 98% pure cell types.⁴¹ Testing for an antifertility effect of immunization with purified hyaluronidase has continued. However, results indicate that circulating antibodies may not be directly responsible for the decreased fertility. It is suggested that inhibition occurs through a cellular immune system; e.g., immune lymphocyte-sperm interaction, possibly resulting in release of spermatotoxins.⁴²

It has been recognized for many years that azoospermia could be induced in the human male with androgens. The need for continuous injections and high dosages to maintain elevated plasma levels has made this approach unattractive. This prompted the investigation of the efficacy of polydimethyl-siloxane implants filled with testosterone to create azoospermia, without elevating plasma testosterone and causing hypertrophy of the accessory sex organs. Although the desired results were achieved in rabbits, the results in adult rhesus monkeys have been disappointing. At a dose that caused moderate increases in prostate and seminal vesicle weights, none of the male rhesus monkeys was azoospermic. These studies are incomplete, but do suggest that testosterone does not suppress pituitary gonadotropin secretion as effectively in male monkeys as in rabbits.⁴³

Mechanism of Hormone Action:

The development of techniques for studying hormone-tissue interactions has brought a clearer understanding of the steps by which target cells respond to their respective specific hormones. The cellular component which interacts first with the hormone has been termed the hormone binder or receptor. For estrogens, progestins and androgens, such receptors have been identified both in the cytoplasm and the nucleus of the respective target cells.⁴⁴⁻⁴⁷ There exists substantiating evidence that the interference with binding prevents the expression of the biological activity of the hormone. Theoretically, if the expression of gonadal hormone action can be prevented in critical tissues of the reproductive system, then infertility could be induced in both male and female animals. Purified receptor systems have now been isolated and have been utilized as model assay systems for the study of hormonal antagonists; these studies call for extreme sophistication. Studies on steroid receptors represent fundamental basic science which could provide significant leads and measures in the search for new antifertility methods.

Studies on gonadotropin receptors, in both male and female gonadal tissues, especially those for LH and hCG, have resulted in a number of significant observations. The LH-hCG receptor has been isolated from rat ovaries and bovine corpora lutea.^{48,49} The receptor appears to be a lipoprotein and in its purified form retains its gonadotropin-binding characteristics. Antibodies to the receptors have been shown to block gonadotropin uptake.

Surprisingly, anti sera to the receptor cross-react with extracts of liver, spleen and kidney tissues made with Triton X-100.⁴⁸ Whether or not this indicates that other non-gonadal tissues have receptor-like proteins for gonadotropins is presently unclear, but it does indicate clearly that this line of investigation is in an extremely early phase, and will require considerable further work.

One of the "spinoffs" from the above studies on gonadotropin receptor has been their application in an assay system for measuring gonadotropins in serum. Thus, the receptor system can be used for the detection of pregnancy in the human and other species.^{48,49} The absolute sensitivity or specificity of this system is not yet established, but it does appear to detect low levels of gonadotropin-like substances in the rabbit blastocysts. Observation of the presence of gonadotropins in blastocysts, that is, prior to implantation, could constitute a major advance in reproductive physiology and biochemistry, but at this time will require stringent confirmation in many species. Another area of research which will be facilitated by the studies of gonadotropin receptors involves the exploration of chemically-modified gonadotropin molecules.^{21,48} The aim of such studies would be to develop inert molecules which could occupy the receptor site and which would prevent the active native gonadotropin from exerting its function on the gonadal tissue.

Similar studies in the steroid hormone receptor field have shown that this line of reason has merit. Prior studies have led to the discovery of modified steroid molecules with antiprogesterational activity. A current study in the estrogen field has led to the finding that modified (tri-hydroxy) estrogens, closely related to estriol, have low estrogenic activity in biological systems, but show high affinity binding to the active receptor for estradiol-17 β in uterine tissue.⁵⁰ Details of this study involving the intricacies of the mechanism of estrogen receptor complexes remain to be clarified.

B. Product Development Research

The Branch continues to support programs involving the testing of new and existing compounds for antifertility activity. These products are derived from existing collections of biological substances, as well as from an active synthetic program for new compounds. Studies aimed at the development of methods for permanent and reversible sterilization in males and females are supported. New drug delivery systems, as well as new mechanical and pharmacological devices are part of the present program.

New Drug Development Program

The synthetic drug development program begun in 1971, involves the synthesis and testing of a variety of potential antifertility agents. One goal is to find new and efficacious chemical contraceptives which will have fewer side effects than those currently available for women. An equally important goal is to develop an effective contraceptive which men may use safely and without risk or loss of libido. This year our 30 contracts in the synthetic drug development program cover a variety of types of chemical substances broadly classified as steroids and non-steroids.

The currently available oral contraceptives are composed of two steroidal compounds, a progestational agent and an estrogen. The estrogen component of the pill (ethynyl estradiol) is a potent synthetic estrogen which, at the dose employed, blocks ovulation. It is generally recognized that the side-effects associated with each of the oral contraceptive preparations are due to the inherent hormonal activity displayed by these synthetic estrogenic compounds. For example, the increased risk of thromboembolic disease (thrombophlebitis, pulmonary embolism, and cerebral thrombosis and embolism) associated with the use of oral contraceptives is related to the dose of the estrogen used, although the quantity of estrogen alone may not be the sole factor involved.

With the above considerations foremost in mind, the contracts involving the synthesis of novel steroids have been undertaken with the purpose of obtaining compounds which show antifertility activity but which will show reduced undesired hormonal effects. The value of a compound with a favorable ratio of antifertility to estrogenic activity would be to provide a contraceptive agent with lesser undesirable side-effects than those currently attributed to the estrogenic component of "the pill". In terms of trying to achieve such a separation of antifertility activity from other hormonal effects, one study on silicon-containing steroids⁵¹ has currently advanced to the primate level of testing after successful demonstrations in lower species such as rats, hamsters and rabbits, of highly favorable biological activity. Two compounds in this series, where the ethynyl group of ethynyl estradiol is replaced by certain trialkylsilylethynyl groups, have shown a 7-fold increase in antifertility potency in rats, orally, when compared with ethynyl estradiol, but show only 20-40% of the estrogenic activity. Preliminary results indicate that one of the compounds is less estrogenic in the monkey than is ethynyl estradiol. These compounds are under current evaluation in rhesus monkeys for their antifertility activity.

Another area of considerable contract activity in the synthetic steroid program is the quest for an antiprogestational agent. Such an agent which could be considered as a once-a-month pill would be a completely novel chemical contraceptive. Its mechanism of action would be primarily that of blocking the action of progesterone (the female hormone required for implantation of the fertilized egg and maintenance of pregnancy) in the uterus. In its application in fertility control it could therefore serve as an implantation inhibitor, a menses inducer (which would cause menstruation in those nonpregnant women where the normal monthly bleeding period has not yet occurred) or as an early abortifacient. A variety of novel steroids are being tested in vivo for antiprogestational activity and the results are being correlated with in vitro progesterone-protein binding assays. There are no positive results at this time, but our synthetic program in this areas has recently expanded. In addition, we are investigating some unpublished leads obtained from the National Cancer Institute's screening program.

Whereas "the pill" for the female contains an estrogen which suppresses the pituitary action responsible for release of the ovum (ovulation inhibition), the use of an estrogen or a progestagen in a male pill would have a comparable effect on the male pituitary whereby sperm production is inhibited. Unfortunately, such a pill would also suppress the pituitary hormones responsible for the production of the male steroid sex hormone, testosterone (the virilizing hormone) which is responsible for libido in the male. The program in this area of synthetic steroids is modest but recognizes the need for a sperm-suppressant which would be free from the undesirable hormonal effects described above. An anti-androgen, unaccompanied by effects that reduce male libido, might be a desirable male pill. There are no good leads at present in this area, but compounds from the synthetic program are being tested for anti-androgenic activity. It will also be possible to make preliminary observations on the effect of such compounds on libido in rats as well as evaluate compounds for direct effects on spermatogenesis in the absence of androgenic or anti-androgenic effects.

The non-steroidal synthetic drug development program consists of contracts involving prostaglandin analogs, peptides, other miscellaneous non-steroids, and entities derived from plant sources.

Prostaglandins are cyclic fatty acids found ubiquitously in the animal tissues. Support for contracts on prostaglandin-like compounds is based upon their potential utility as luteolytic agents (chemicals which cause degeneration of the corpus luteum whose function is to secrete progesterone which is essential for the maintenance of pregnancy), or menses inducers for use as once-a-month pills. Natural and synthetic prostaglandins (PGs) have been demonstrated to have luteolytic activity in animals but not in humans. The presently available PGs have a number of side-effects which impose a limitation on their utility in fertility regulation. The justification for our modest but continuing synthetic program in this area is that an agent, luteolytic in the human and devoid of undesirable side-effects (gastrointestinal disturbances) would be a novel and desirable contraceptive agent. An attempt to effect such a separation of activities is the preparation of chemically modified analogs of selected PGs. Several of these analogs show significant antifertility activity (e.g., 13,14-dehydro-PGF_{2α}⁵² is 8 times as active as PGF_{2α} subcutaneously in hamsters

but have much less smooth-muscle activity than the natural PGs. Several of these analogs are currently being synthesized in gram quantities for evaluation in primates. From a technical point of view, this is a considerable achievement.

Luteinizing hormone releasing factor (LRF), which is formed in the hypothalamus is structurally a decapeptide, and forms a vital link in the whole sequence of reproduction. LRF causes the release of both LH (luteinizing hormone) and FSH (follicle stimulating hormone) from the pituitary and thereby controls the function of the ovary and the release of the egg. It has been postulated, therefore, that antagonists to LRF could be developed which could prevent the midcycle surge of LH which causes ovulation and thus lead to a new type of antioviulatory contraceptive, different in concept from the current oral contraceptives. Our contract program in this area is somewhat modest but is also directed toward finding inhibitors or blockers of LRF. A number of such analogs have shown significant LRF antagonist activity in vivo. Currently, one such synthetic antagonist, [des His², des Gly¹⁰]-LH-RH, ethylamide, has been observed to block ovulation in the rat.⁵³ However, its duration of action is too short for practical utilization at this time. Further synthetic work in this area will endeavor to uncover more potent and longer-acting inhibitors.

Our miscellaneous non-steroidal program involves the search for completely nonhormonal-like agents for fertility control in both sexes. There are no relatively safe and effective non-steroidal antifertility agents known. Most of the "active compounds" which were previously reported in rat studies have been found to be either ineffective or unsafe for the human female and unsafe for the human male. Our male program in this area is, therefore, directed towards a safe antispermatogenic agent. Our female program in this area has led to a novel non-steroidal agent (a quaternary ammonium salt) which is completely effective in dogs, orally,⁵⁴ but effective in rats and rabbits only at doses at which gastrointestinal toxicity occurs. Currently, this study is being extended to determine the period of maximum contraceptive efficacy in dogs. A study in non-human primates is being initiated.

The search for antifertility agents from plant sources has just begun. A literature survey has revealed that very few previously reported allegations of antifertility activity from plants are worth pursuing. Efforts in this area are being directed towards a modest screening program of previously untested plants. In addition, we have a single contract which will involve the development and identification of active compounds (blastocidal and spermicidal) from a plant. P. dodecandra, and the confirmation of this reported biological activity.⁵⁵

Testing Facility

Almost 200 compounds have been tested in the past year for antifertility and classical endocrine activities at the CDB testing facility.⁵⁶ Several potential leads have been identified and a significant separation of antifertility and endocrine activities has been demonstrated for several members of one chemical class of compounds (see New Drug Program). Two members of this series are being studied in a primate species (monkeys) to confirm the separation of estrogenic and postcoital antifertility activities observed earlier in smaller animals. Baseline studies on 16 reference standards have been completed and

are being utilized in the design of individual assays for potency of experimental compounds. The contractor continues to cooperate with the staff of the CDB in the development of new and more sophisticated test procedures. These include modifications of postcoital antifertility and antispermatogenic tests as well as the establishment of an in vitro assay for oxytocic activity using the hamster uterus. Data generated by the testing facility is rapidly disseminated by the CDB staff to individual chemists in our contract synthetic program permitting optimal direction for subsequent modifications in compound structure.

Drug Delivery Systems

Research on new drug delivery systems has progressed satisfactorily and a wealth of new information has been acquired. The biodegradable systems for delivery of steroids appear entirely feasible even though the complexity involving their performance is very great.⁵⁷⁻⁶⁰ The initial assumption that biodegradation of the polymer matrix would be the controlling factor for drug release is not entirely correct. Diffusion of the various steroids from the polymer matrix is substantial and thus a sustained and uniform release has become a difficult technical problem. Most of the initial work has been carried out on copolymers of lactic and glycolic acids.^{57, 60} It has been observed that the presence of 20% or more of glycolide in the copolymers results in a rapid degradation of the system and an unsatisfactory drug release rate. Homopolymers of L-lactic acid have also been found unsuitable because of their exceedingly slow degradation and poor drug diffusion characteristics. Polymer made of dl-lactide shows promise both in vitro and in vivo studies utilizing dogs as the animal model. Some promising work has come from studies of polypeptide polymers where the degree of degradation can be controlled by changes in amino acid composition.⁵⁸ The polymers utilizing lactides, glycolides and amino acids are theoretically degraded to normal body constituents and thus the body's disposal of the breakdown products presents no problems.

A promising advance was made in the formulation of a polymer made of ϵ -hydroxycaproic acid. This polymer, although it degrades rather slowly, permits rapid diffusion of steroidal drugs. In this respect it is comparable to silastic polymers with the added advantage of possible biodegradability.⁵⁹

Work progresses in non-biodegradable drug delivery systems. The contraceptive activity of estriol in an intrauterine device has been assessed in the rabbit. The presence of an estriol-releasing IUD in one horn of the uterus showed good antifertility activity, whereas the horn containing an inert IUD (control) had numerous implants. The action of the drug occurs at a dose level (1.5 μ g/day) which is considerably below that required to produce an effect via a systemic route. This practical area of research warrants continued support and possibly further expansion. Another system which is being developed is for the sustained delivery of silver ions.⁶² It is based on the observation that the application of silver nitrate to the fallopian tubes results in epithelial damage and the subsequent occlusion of the tube. Since the direct application of silver nitrate as a cream formulation is difficult to control and the results are consequently somewhat unpredictable, it is desirable to have a model for delivering the silver ions in a controllable fashion. It appears that

such a system has been achieved. It consists of a biodegradable matrix which is capable of releasing silver ions at a controlled rate over a period of 24-48 hours. The utility of the system is presently being assessed in laboratory animals.

Development of Sterilization Techniques (Female)

Sterilization by surgical intervention on the fallopian tubes is becoming increasingly popular with American women as a means of contraception. The CDB is supporting the development of nonsurgical approaches to female sterilization through the use of tubal occlusive devices and the instruments required for their placement directly into the oviducts.^{63,64} An improved steerable hysteroscope has been developed which permits the insertion of occlusive plugs into the uterine ostia of the oviducts under direct visualization. The instrument has been successfully employed in the identification of the openings of the oviducts into the uterine cavity, the ostia in intact baboons and in extirpated human uteri.⁶³ Clinical trials are presently underway in patients scheduled for hysterectomy.

Several type of soft occlusive devices have been designed and fabricated which incorporate mechanical aids meant to improve the retention of the devices. These are being evaluated in the baboon animal model prior to clinical trial. Another transcervical approach to female sterilization is the blockage of the fallopian tubes by a silastic elastomer which cures in situ.⁶⁴ A clinical prototype instrument for the delivery of a precise volume of the liquid elastomer to the uterotubal junction has been developed. Known as a cornu-salpingoguide, this instrument is first seated blindly in the uterine cornu; this is followed by extrusion of the elastomer which then cures into a soft plastic within the fallopian tube. Studies demonstrating the safety and utility of this method have been completed in rabbits and monkeys. Protocols for the first clinical trials of the cornuosalpingoguide in patients undergoing hysterectomy are being reviewed.

Development of Sterilization Techniques (Male)

The development of devices which produce a reversible vasectomy is an important part of our current program, and observations to date continue to encourage and support the feasibility of the concept. Four types of occlusive devices are undergoing investigation: i) polyurethane devices coated with a microporous interface⁶⁵; ii) all soft-silicone-rubber shuttle-stem valve, with Dacron velour-coated flanges.⁶⁶ iii) all-gold-and-stainless steel with ball valve⁶⁷ iv) silastic-silicone rubber-covered with dacron fibers⁶⁸

Evaluation of these devices has focused primarily on biological aspects to determine which factor influence (a) sperm transport and motility; b) surgical insertion procedures; c) tissue ingrowth and tissue reaction; and d) to determine the success with which these devices can be cycled through "open" and "closed" stages. Devices implanted into the vas deferens in guinea pigs in either the "on" or "off" positions and then reversed, showed aspermia or sperm, as the case may be, in the ejaculate, thus confirming the feasibility of reversibility.⁶⁷ In dogs, sperm production is maintained for extended periods of time when devices are implanted in the "on" position; sperm transport resumed when devices were implanted in the "closed" position

and later opened. Restoration of fertility, however, has not as yet been demonstrated. ⁶⁶

Results from studies on absorbable intravasal stents have been encouraging. Of particular interest are the polyester stents, which are completely re-sorbed in the vas deferens of the guinea pig within two weeks.⁶⁸ After an average period of 8.5 weeks, animals reanastomosed with the polyester stents showed sperm counts and sperm motility which were essentially unchanged from their preimplant values. Successful matings have occurred with polyester-vasovasotomized males, indicating no deleterious effects of the procedure on sperm.

Clinical Studies (Female)

The safety and efficacy of ethynyl estradiol and conjugated natural estrogens as postcoital contraceptive agents are being studied under uniform protocols at several institutions. ^{69, 72} Five mg daily of ethynyl estradiol or 30 mg daily of conjugated natural estrogens are being administered to patients requesting this form of contraception and who meet rigid criteria for acceptance into the program. The drugs are taken for 5 consecutive days starting no later than 72 hours following unprotected midcycle coital exposure. Each subject receives a pre- and post-treatment medical examination and pregnancy test. Both drugs appear efficacious in preventing pregnancy but unpleasant side-effects such as nausea and vomiting are common at these dosages. No serious untoward reactions have been reported. The accumulation of patient-use-effectiveness data is still in progress.

The initial clinical trial of a fluid-filled, soft intrauterine device (IUD) has yielded encouraging data.⁷³ The IUD has been inserted into 77 subjects, 69% of whom were nulliparous, for a total of 350 "women-months" of use. Additional subject experience is available from non-federally supported studies of insertions for a total of 570 subjects and 3150 "women-months." The average length of individual participation in the project has been 6 months, which is too short to permit meaningful statistical analysis using the life-table method and computation of continuation rates. However, certain qualitative observations regarding trends can be made. Removals are the most important occurrence and account for more than half the number of withdrawals from the studies. Removals for spotting and bleeding are more than three times as numerous as those associated with pain and cramps. Expulsions are the next most frequent event. There have been a total of four pregnancies with the device in place. Comparison of the present preliminary data with the much more extensive data available for other IUDs reveal a characteristic pattern of improved performance for the fluid-filled IUD. The projects will be expanded to afford a better basis for performance-evaluation.

Clinical Studies (Male)

The clinical trial of Danazol together with various esters of testosterone as a male antifertility agent has been completed and the volunteers are presently in the post-treatment phase.⁷⁴ The trial involved 92 male volunteers who were subdivided into several treatment groups. The following conclusions can be drawn from this project; i) Danazol by itself has only a marginal effect on

sperm production; ii) Danazol together with methyl testosterone on a daily basis was marginally effective; iii) Danazol together with a once-a-month injection of testosterone enanthate produced the sharpest decline in sperm production.

The study has clearly indicated that methyl testosterone has a limited utility in male fertility regulation. Furthermore, the presently available formulation of Danazol is very poorly absorbed from the gastrointestinal tract and thus the daily dose (600mg) is by necessity very large. Even with the most efficacious treatment (Danazol plus testosterone enanthate), suppression of spermatogenesis was not complete and there was a significant lag period (3-4 months) between initiation of therapy and ultimate suppressions of sperm. On the positive side, it must be stated that there is no problem in securing volunteers for male antifertility studies; there were no toxicological manifestations or other undesirable side-effects. Further research in this area will be concerned with devising more adequate treatment schedules and/or more effective drug combinations.

Hormone Distribution

At the present time the CDB, in cooperation with the NIAMDD, is supplying the scientific community with samples of hCG, hCG subunits and LRF, all of which were produced through CDB sponsored research contracts. Distribution of ovine LH and its subunits will be initiated as soon as these materials are properly characterized.

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43. Ewing, L. L., N01-HD-3-2745, \$64,256. Androgen polydimethylsiloxane implants: Metabolic fate of testosterone and contraceptive efficacy of different androgens. The Johns Hopkins University
44. Bardin, C. W., N01-HD-1-2228, \$83,519 (2 years). Assay of progesterone receptor activity in the guinea pig uterus. Milton S. Hershey Medical Center
45. Toft, D. O., N01-HD-3-2769, \$111,121 (2 years). Studies on steroid hormone receptors and their role in reproductive biology. Mayo Clinic

46. Stavely, H., N01-HD-2-2710, \$73,445. Progesterone-binding proteins as a tool for detection of progesterone antagonists and agonists. Institute of Medical Research, Toledo Hospital
47. Jensen, E. V., N01-HD-9-2108, \$108,423 (2 years). Estrogen-receptor substances of oviduct tissue. University of Chicago
48. Ryan, R., N01-HD-1-2232, \$59,740 (2 years). A model for studying inhibition of LH action on the corpus luteum. Mayo Clinic and Foundation
49. Saxena, B. B., N01-HD-2-2763, \$45,377. Biochemistry of and mechanism of action of gonadotropins. Cornell University
50. Wotiz, H. H., N01-HD-3-2787, \$57,572. Impeding estrogens as contraceptives-mechanism of action. Boston University School of Medicine
51. Tanabe, M., 71,2462, \$70,000. The synthesis and testing of anti-fertility compounds. Stanford Research Institute
52. Fried, J., 71-2117, \$137,968 (two years). Synthesis of prostaglandin analogs for biological studies. University of Chicago
53. Schally, A. V., 72-2741, \$90,000. Production of new type of contraceptives based on derivatives of luteinizing hormone-releasing hormone (LH-RH). Tulane University School of Medicine
54. Dalgard, D. W., 72-2717, \$49,916. Study of antifertility activity of antibiotic mixture in animals. Hazleton Laboratories, Inc.
55. Stolzenberg, S., N01-HD-4-2833, \$45,588. Blastocidal and spermicidal action of extracts and compounds from Endod (Phytolacca Dodecandra). Stanford Research Institute
56. Rosenkrantz, H., 72-2746, \$969,723 (2 years). Biological testing facility for the evaluation of new antifertility drugs and devices. Mason Research Institute
57. Nuwayser, E., N01-HD-3-2738, \$254,856 (2 years). Implantable bio-absorbable capsules as slow-release contraceptive drug delivery systems. Abcor, Inc.
58. Sidman, K. R., N01-HD-4-2802, \$60,900. Development and evaluation of a biodegradable drug delivery system. A. D. Little, Inc.
59. Wall, M., N01-HD-3-2741, \$299,994 (2 years). A study of the use of biodegradable polymers for the sustained delivery of contraceptive drugs. Research Triangle Institute.
60. Wise, D., N01-HD-3-2736, \$245,972 (2 years). Development and testing of an implantable contraceptive delivery system. Dynatech R/D Company

61. Dnowski, P., N01-HD-3-2739, \$133,000. Intrauterine release of estriol for contraception. Michael Reese Hospital
62. Gregor, H. P., N01-HD-3-2797, \$86,600. Polyelectrolyte delivery systems for chemical cauterization and sustained release. Columbia University
63. Brueschke, E., 72-2233, \$483,769. Development of an intrauterine sterilization system for the female. ITT Research Institute.
64. Erb, R. A., 72-2237, \$27,694. Device and technique for blocking fallopian tubes. Franklin Institute.
65. Robinson, T., N01-HD-2-2796, \$99,152. Development of a reversible vas-occluding prothesis utilizing a microporous tissue interface. TECNA Corporation.
66. Brueschke, E., N01-HD-1-2229, \$189,694. Design and development of implantable contraceptive devices for use in the male. IIT Research Institute
67. Freund, M., N01-HD-1-2223, \$68,623. The use of reversible vasectomy device in guinea pig. New York Medical College
68. Nuwayser, E., N01-HD-1-2227, \$175,637. Development of new sterilization techniques. Abcor, Inc.
69. Hanson, M., 73-2762, \$56,662 (2 years). Clinical studies of estrogens as postcoital contraceptive agents. Planned Parenthood of Minnesota
70. Hale, R. W., 73-2760, \$10,400 (2 years). Clinical studies of estrogens as postcoital contraceptive agents. Kapiolani Hospital
71. Pruett, K., 74-2809, \$132,342 (2 years). Clinical investigation of ethynylestradiol and conjugated natural estrogens as postcoital contraceptive agents. Planned Parenthood League of Connecticut
72. Bard, D. S., 73-2761, \$53,086 (2 years). Study of the safety and efficacy of a conjugated estrogen preparation as a postcoital contraceptive. University of Florida
73. Kitrilakis, S., N01-HD-3-2767, \$51,980. Clinical evaluation of a fluid-filled intrauterine contraceptive device. TECNA Corporation.
74. Paulsen, C. A., N01-HD-1-2234, \$58,315. Contraceptive development studies for males. University of Washington

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Detection of Ovulation in Chronically Instrumented Animals
Contractor: University of Washington
Money Allocated: \$45,600 (FY 72), \$118,308 (FY 73)

Objectives: The objective of this research is to devise a technique for sensing the arrival of the cumulus mass in the ampulla. The arrival of the cumulus in the ampulla so closely follows the rupture of the ovarian follicle that the detection of such an event would serve as detection of ovulation. The research would be initially performed employing acute (open dish) preparations and subsequently chronic preparations would be employed. The initial objective was to utilize the ultrasonic transduction as a method for cumulus mass detection. Because of a number of technical problems with ultrasound, the use of optical transducers is being investigated.

Major Findings: The contractor has developed optical transducers which can pick up the passage of the cumulus mass through the ampulla. The method is based on the fact that cumulus masses modulate the optical transparency of oviduct with a resultant increase in light transmission. The assembly consists of a basic cuff which contains on one side a small red-light-emitting diode (LED) and a phototransistor (PT) on opposite sides of the cuff. Utilizing such a system in chronically implanted rabbits, they were able to pick up 90% of the ovulated cumulus masses. In the case of stained masses, these showed up on the record as decreases in optical transmittance, whereas the unstained masses increased transmittance. Changing of the optical cuff's location toward the fimbria improved the signal to noise ratio. The rabbit ampulla being quite translucent allowed for a visual observation of the cumulus mass movement and a direct comparison with the transducer signal. Acute experiments confirmed the validity of the signal.

Acute experiments with the monkeys proved to be somewhat more difficult. Oviduct wall thickness reduced the amplitude of the signal. Likewise, direct observation of unstained cumulus masses can not be done. With the stained masses, a characteristic decline in optical transmittance was observed. This could be substantiated by direct visualization of the stained cumulus passage. Initial experiments with the unstained cumulus masses likewise produced a decrease in transmittance, which was contrary to the observations made on rabbit oviducts. Subsequent experiments employing more refined techniques have resulted in a consistent increase in light transparency. The refinement was related to the placement of the transducer on the first ampullary segment, 7-10mm from the ostium. The average amplitude of the unstained cumulus mass signals was approximately one half that of the stained cumulus mass signals. Frequently, contractions appear to contribute "noise" to the signal either just preceding or during the passage of cumulus masses, but the cumulus mass signal may be easily extracted with conventional filtering techniques since the frequency domains of the "noise" and cumulus mass signal are substantially different. The overall impression from the present

experiments is that the placement of the cuffs on the oviduct may be a critical variable.

The presently available optical transducers do not interfere with normal reproduction of instrumented rabbits.

Significance to Biomedical Research and Program of the Institute: Accurate determination of the time of ovulation is of primary interest to the program of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of Techniques for the Detection of Ovulation
in the Primates
Contractor: Illinois Institute of Technology Research Institute
Money Allocated: \$83,035 (Fy 73)

Objectives: The objective of this research is to develop a simple assay system for impending ovulation based on changes in the chemical nature of vaginal fluids. The research is based on the hypothesis that high levels of estrogens that are observed prior to ovulation change the metabolic pattern of the vagina and that a careful study of metabolic products may reveal a predictable pattern useful for ovulation ascertainment.

Major Findings: The contractor has developed gas chromatographic procedures for the identification and quantification of volatile fatty acids in vaginal emanation. Preliminary results from a small sample of normal women volunteers indicate that there exists a peak of volatile fatty excretion prior to the rise in basal body temperature which normally occurs following ovulation. No definitive conclusions can be made at this time since the data are somewhat equivocal.

Significance to Biomedical Research and Program of the Institute: Development of simple and reliable methods of ovulation detection which can be useful in fertility regulation is directly related to the stated objectives of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new fertility regulating methods and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of Techniques for Study of Oviduct Transport and Detection of Ovulation in Primates
Contractor: Case Western Reserve University
Money Allocated: \$123,026 (FY 72) two years

Objectives: It is proposed to investigate the possibility of developing microelectronic sensors and radio-telemetry systems which can be totally implanted in the experimental animal to record several selected parameters believed to be related to ovulation. The proposed telemetry system will measure transovarian impedance, ovarian tubal impedance, ovarian size, ovarian temperature, and body core temperature. Other parameters, such as pressure, tubal motility and the electrical potential, could be telemetered with the same systems. The electrical impedance measurements are unique and have not been previously studied for the ovary, although the method has been successfully used in detecting changes in anatomical structure and histologic changes such as edema and hyperemia. It is believed that the transovarian electrical impedance changes will be easily seen through the ovarian cycle due to follicular growth and atresia. The follicular fluid in the growing follicle is isotonic with serum and therefore a good electrolyte so that the ovarian impedance will be low at the time of ovulation. During ovulation, when the follicle ruptures, there should be a sharp increase in electrical impedance. Following ovulation, when the ruptured follicle forms a corpus luteum, there should be a second increase in impedance. Correlative measurements of gonadotropin levels and basal body temperatures will be the indices of ovulation. In addition, measurement of ovarian temperature as compared to body core temperature will be carried out and it is expected that the differential between these two temperatures will increase during the follicular stage to the point of ovulation, whereupon the body temperature will rise, reducing the differential. For this project it is proposed to use telemetry systems previously developed in this laboratory and to modify them with special attention to the design and modification of transducers for this project.

Major Findings: Instrumentation designed to measure impedance changes of the oviduct and the uterus has been evaluated in animals. Changes in contractility effect impedance. Because of the small size of the impedance electrodes a number of them can be placed on an organ without interference with function. The contractor was not successful in accurately determining ovulation time by measuring thermal conductivity changes or impedance changes.

Significance of Biomedical Research and Program of the Institute: Accurate determination of the time of ovulation is of primary interest to the program of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: An Interdisciplinary Program Project on the Biology of the Oviduct and Gamete Transport
Contractor: University of Washington School of Medicine
Money Allocated: \$192,490 (FY 70 Funds), \$177,734 (FY 71 Funds), \$177,734 (FY 72 Funds), \$402,801 (FY 73 Funds for Two Years)

Objectives: This is a comprehensive interdisciplinary program project on the basic biology, physiology, and endocrinology, of oviductal function in rabbits and monkeys. Seven different specific aims are enumerated: (1) the development of techniques for direct observations of the normal function of the various segments of the oviduct in situ; (2) development of miniature sensing devices and telemetry equipment that will permit study of the normal activity of the oviduct in unanesthetized, unrestrained, rabbits and monkeys; (3) study the electrophysiological characteristics of oviductal musculature; (4) investigation of intrinsic innervation, pharmacological responses, and the possible role of pacemakers in oviduct function; (5) ultrastructural studies of the epithelium and musculature under different physiological conditions; (6) development of techniques of in vitro cultivation of oviductal tissue as a potential bioassay system; and (7) studies of the mechanism of sperm transport through the oviducts.

Major Findings: The ciliary current in oviducts of pig-tailed macaques and humans was consistently toward the uterine end; this is unlike the observation in the rabbit and the sow. Transport of spermatozoa within the oviducts of these species is most likely dependent either on flagellations of sperm or muscle activity. The rate of particle transport is considerably slower in monkey and human oviducts than in those of the rabbit, pig, cow and sheep.

Electro-physiological studies of the mesotubarium superius of the rabbit oviduct have confirmed the earlier findings that acute estrogen withdrawal accelerates muscle contraction in the reproductive tract.

An in vitro rabbit oviduct preparation has been established. Muscle activity and cumulus mass transport have been monitored. This isolated oviduct preparation may prove useful in delineating various aspects of gamete transport.

Cumulus mass transport through the oviducts of ovulated monkeys was significantly faster than in animals during the preovulatory period.

New observations on sperm transport mechanisms in the isthmus of the pig and the rabbit have been expanded. These show that transport of the luminal contents of the isthmus to the site of fertilization occurs in less than 6 seconds. During the luteal phase of the cycle, the transport mechanism reverses so that the eggs are slowly transported toward the uterus.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the various functions of the oviduct which are responsible for gamete transport and survival, fertilization, and transport of the fertilized egg to the uterus.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the Contraceptive Development Program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : A Bioengineering Approach to the Study of Tubal Activity and Contraception
Contractor: Drexel Institute of Technology
Money Allocated: \$116,456 (FY 69), \$38,437 (FY 70), \$7,688 (FY 71), \$64,893 (FY 72), \$111,742 (FY 73), \$8,100 (FY 74)

Objectives: The objective of this program is to determine patterns of oviductal motility at various stages of the menstrual cycle, the influence of exogenous hormones on this pattern and the contribution of oviductal contractions to gamete transport. Inherent in these objectives is the development of suitable transducers and telemetry equipment to permit the uninterrupted acquisition of data in unrestrained monkeys.

Major Findings: The contractor fabricated a number of implantable transducer and telemetry devices in final form for physiological studies in monkeys. These instruments had been designed, developed and calibrated during the preceding contract periods.

Each implant is of a two channel telemeter whose data output is recorded 24 hours a day both on hard copy and magnetic tape. Estrogen and progesterone are determined three times per week to monitor the stages of the menstrual cycle including an estimation of the time of ovulation. Thirty-one monkey months of contractile data representing 17 ovarian cycles of various experimental design have been collected. This data is being analyzed through a specially designed computer program in an effort to correlate the duration, amplitude and force of contractions as well as the inter-contraction interval with the physiological status of the animal.

The initial analyses of data available at this time indicate very general trends. Estrogen domination, for example, is associated with a tendency to order and rhythmicity of contractions while under progesterone dominance a loss of periodicity, amplitude variability and a general decrease in the frequency of contractile events.

Significance to Biomedical Research and Program of the Institute: This work may help to explain the role of oviductal motility in the reproductive process and the manner by which modifications in the normal sequence of estrogen/progesterone ratios alters fertility. As such, this project is directly related to the stated goals of the Contraceptive Development Branch.

Proposed Course: It is anticipated that the contractor will complete the acquisition and analysis of data originally proposed within the next contract period. No further support beyond an additional 18-month period is planned.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Regulation of Bicarbonate in Oviductal Fluid
Contractor: Johns Hopkins University
Money Allocated: \$50,656 (FY 73)

Objectives: The objective of the proposed project is to determine whether pharmacological regulation of bicarbonate levels in oviductal fluid could serve as the basis of a new contraceptive method. The rationale for this approach is as follows: Since the oviduct actively secretes and maintains high levels of bicarbonate, and since bicarbonate has important roles in early reproductive processes, it is possible that drugs can be found which will alter bicarbonate concentrations sufficiently to interfere with conception. Accurate and sensitive micro-analytical methods have been developed and will be used to determine whether hormones and drugs influence bicarbonate levels in oviductal fluid.

Major Findings: Oviductal fluid was collected over a one-to-two-hour period from both oviducts of rabbits anesthetized with pentobarbital. For all collection periods, the concentrations of Na^+ , K^+ and HCO_3^- (total CO_2) of oviductal fluid were higher than plasma. The concentration of Cl^- of oviductal fluid was similar to plasma and the concentration of H^+ and glucose was lower than plasma.

The actual distribution ratio of the concentration in plasma averaged 1.80 and was not significantly different during day 0, day 1 and day 2. The average transmembrane potential (PD) was 10.8 mV (lumen negative) during this period. Calculation of the theoretical distribution ratio, based solely on the influence of electrical forces, gives a value of 0.67. Thus, the actual distribution ratio 1.80, is greater and significantly different from the theoretical ratio, 0.67, and suggests there are active transport mechanisms for the accumulation of bicarbonate during estrus and the first two days of pregnancy. In addition, these mechanisms appear to operate at the same rate to maintain bicarbonate levels constant during the days studied.

Preliminary work with acetazolamide, a potent carbonic anhydrase inhibitor does not suggest that this inhibitor can reduce the accumulation of bicarbonate in oviductal fluid. This finding indicates either that the carbonic anhydrase of the oviduct is not being inhibited by acetazolamide, or that the accumulation of bicarbonate is not dependent on carbonic anhydrase activity.

Significance to Biomedical Research and Program of the Institute: Bicarbonate has been established as an essential element in fertilization and embryo development. Therefore, its control is clearly relevant to the goals of the Contraceptive Development Branch.

Proposed Course: Discontinuation. The principal investigator is relocating.

NICAD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Physiology of the Oviduct
Contractor: Stanford University Medical School
Money Allocated: \$91,585 (FY 70), \$10,000 (FY 71) \$141,864 (FY 72)
\$112,458 (FY 73)

Objectives: The objective of this investigation is to develop electrodes, transducers and other instrumentation for study of electrical, mechanical, pressure, and flow phenomena and to use these instruments to investigate the functional properties of the muscle coat of the oviduct (1) as small segments in the tissue bath, (2) in isolated organs and (3) in the intact subject. By determining changes in properties of the oviductal muscles as a function of hormonal environment, knowledge will be sought as to the mechanisms which control oviduct function.

These studies will involve measurements of the electrical and contractile behavior of oviductal tissue using excised oviducts of rabbit and man, at various stages of the reproductive cycle. Studies of pacemaker activity, the interrelationship of electrical activity from one area to another, correlation of electrical and mechanical activities, and intraluminal pressure changes will be conducted. The final phases of the study will involve the measurement of these functions in intact, free-roaming animals and hopefully, eventually in women. All data will be programmed for computer analysis and a mathematical model of the system will be undertaken similar to one the investigator has developed for intestinal smooth muscle.

Major Findings: Sources of force transducer fragility have been isolated and it is anticipated that proper attachment of highly flexible wires will be accomplished. Good progress has been achieved in the development of pressure transducers.

Patterns of in vivo oviductal activity have been recorded from conscious rabbits before and after ovulation. Analysis of contractility via power spectra reveal that activity of the isthmic portion of the oviduct after ovulation is spectrally pure with fast and stable contractions approaching a pure sinusoid.

The small size of the force transducer makes it potentially useful for application to a variety of tissues.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to encourage scientists from other disciplines to apply their unique skills to studies of reproductive function.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Oviductal Fluid in the Rat and Mouse: A Study of the Chemical and Physical Properties and Mechanism of Formation
Contractor: The Johns Hopkins University
Money Allocated: \$46,014 (FY 73), \$60,982 (FY 74)

Objectives: The objectives of this research are: (1) to develop methods for the in vivo collection of oviductal fluid from the rat and mouse; (2) to establish the micro- and ultra-micro chemical techniques required for the analysis of the oviductal fluid; and (3) through the use of these techniques, to determine the chemical and physical properties and rate of formation of oviductal fluid throughout the estrous cycle, and to evaluate the influence of a changing hormonal environment on the formation of oviductal fluid in the two species.

Major Findings: A method for the collection of oviductal fluid from the rat has been developed, and in conjunction with this, methods for determination of the pH of oviductal fluid and determination of the electrical potential difference across the oviductal epithelium. The initial results indicate that (1) the oviductal epithelium is capable of maintaining a pH gradient between the oviductal lumen and plasma. In estrus, the oviductal fluid is alkaline with respect to blood; (2) the oviductal epithelium is capable of maintaining a gradient for chloride between the oviductal fluid and plasma; (3) a potential difference exists across the oviductal epithelium, lumen negative with respect to the abdominal cavity. Although the magnitude of the potential difference is currently in question, its presence is a factor which must be considered when evaluating the forces affecting the distribution of electrolytes across the oviductal epithelium.

Significance to Biomedical Research and Program of the Institute: The practical implications of the results of this approach are clearly related to the development of contraceptive methods based on alterations of oviductal function and thus to the stated goals of the program.

Proposed Course: This is expected to be a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Morphochemical Analysis of Oviductal Mucins
Contractor: University of Minnesota
Money Allocated: \$17,763 (FY 70); \$19,395 (FY 71); \$17,523 (FY 72);
\$28,929 (FY 73)

Objectives: The investigator proposes to study further oviductal mucins in the pre- and postcoital rabbit oviduct by autoradiographic procedures for determining the chemical composition of the carbohydrate portion of the mucins. An autoradiographic study of the uptake and subsequent localization of the possible precursor sugars of oviductal mucins will be attempted on a time course basis utilizing certain labeled neutral and amino sugars. The S³⁵-sulfate radioisotopic label, which is the only substance known to be incorporated into oviductal mucin has been followed in a similar manner following injections of isotope under the postcoital hormonal condition.

Major Findings: Cytochemical staining patterns in precoital oviducts revealed the regionality of mucins, namely, that the ampulla and isthmus contained highly acidic moieties in their mucous components. Based on histochemical staining patterns and alterations of such staining by specific chemical blockage and enzymatic removal, these acidic carbohydrates were thought to contain sulfate in the ampullary and isthmian portions of the oviduct. The localization of radioactive sulfate in mucins in these two portions of pre-coital oviduct can be detected in saccules and dilated cisternae of the supranuclear Golgi zone in the secretory cells of ampulla and isthmus from 30 sec. to 5 min. Subsequently, labeling can be observed in larger secretory granules of these mucinous cells, and then, all labeling is confined to extracellular sites, particularly on the cell surface by one hour. Staining of mucins by the combined basic dye procedure consisting of high iron diamine followed by Alcian blue has revealed the presence of sialic acid-rich secretions in the precoital isthmus. These results confirm the biochemical viewpoint that most disaccharides forming glycoproteins are coupled to sulfate in the Golgi zone and/or endoplasmic reticulum. The specific chemical removal of sulfate by active methylation confirmed the presence of sulfated carbohydrate in ampullary and isthmian mucins of rabbit precoital oviduct.

S³⁵-sulfate is largely present in the secretory cells of the ampullary and isthmian portions of rabbit oviduct from 6 to 24 hrs. postcoitum. These preliminary results are in accord with previously reported results on the incorporation of S³⁵-sulfate in precoital tissues. The intracellular pattern of radioisotopic localization is present in secretory granules at early post-injection times rather than in Golgi components as observed in precoital tissues.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the oviduct.

Proposed Course: Discontinuation. The investigator has withdrawn his renewal application.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Genetic Expression in Mammalian Embryo
Contractor: University of Colorado Medical Center
Money Allocated: \$26,197 (FY 70), \$26,197 (FY 71), \$32,344 (FY 72)
\$35,905 (FY 73)

Objectives: The objective of this investigation is to study RNA synthesis as a function of hormonal environment in both the embryo and the oviduct during the early stages of environment in the rabbit.

Major Findings: Biochemical and radioautographic data show that protein synthesis is increased markedly at the morula stage of rabbit development (60 hour embryo). In the late morula, an increase in cytoplasmic ribosomes is observed, suggesting that ribosome availability may be rate-limiting for protein synthesis during cleavage.

Incorporated ^3H -amino acids become highly localized within the nucleoli of late morulae that have been pulse-labeled for 10 minutes. This localization suggests that ribosomal protein synthesis is increased at the same time that ribosomal RNA synthesis has been shown to increase. Changes in both the incorporation of ^3H -amino acids and cytoplasmic ribosome density were found to occur "synchronously" in all embryonic cells during the cleavage and early blastocyst period (84 hours of development). Between 84 hours and 108 hours considerable differences in the number of ribosomes per unit area of cytoplasm appear among the cells of the blastocyst.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the hormonal control of intra-cellular processes.

Proposed Course: Discontinuation in favor of grant support.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Immunopharmacology of the Human Fallopian Tube
Contractor: Stanford University
Money Allocated: \$40, 793 (FY 70), \$79,881 (FY 71), \$91, 602 (FY 72),
\$270,622 (FY 73), for two years

Objectives: The work to be carried out under this contract involves two aspects of oviductal physiology: (1) characterization of the permeability and transport characteristics of oviductal epithelium; and (2) characterization and quantitation of the oviductal response to the anaphylactic reaction between an antigen and the specific antibody bound to oviductal cells. Work will be carried out in laboratory animal and human oviductal tissue, in vitro.

Major Findings: Studies of human tissues continued to reinforce previous observations that tissues of progesterone-dominated hosts have up to 3-fold greater resting histamine content than tissues of estrogen-dominated patients.

Progesterone-dominated tissues are significantly leakier than estrogen-dominated human oviducts. The spontaneous release of mediator into balanced salt solution was 3- to 6-fold greater in the former case than in the latter.

In the guinea pig progesterone injection increased the resting histamine content 130% and estradiol decreased it 72%. With respect to releasable histamine progesterone reduced the specifically releasable fraction by 40% whereas estrogen increased the antigen-activated release by 300%.

Steroids do influence antibody production in the female guinea pig. In the estradiol treated animals the decay curve for antibody titers was much less pronounced than in progesterone and testosterone treated animals. The hormone effects seen in the female animal were not observed in males.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support innovative studies on the physiological and pharmacological properties of laboratory animal and human oviductal tissue.

Proposed Course: During the forthcoming year the stated objectives of this contract will be fulfilled.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Oviduct Cilia: Structure, Function, Development
Contractor: University of California
Money Allocated: \$34,343 (FY 70), \$9,097 (FY 71), \$35,038 (FY 72)
\$29,138 (FY 73), \$30,634 (FY 74)

Objectives: The stroke form and fine structure of oviductal cilia is to be explored using techniques previously applied to mussel gill cilia. The aim is to provide information on the beat mechanism of oviductal cilia and to evaluate the influence of various agents on the mechanism. Techniques to be employed in these studies include high-speed cinematography, electron microscopy, and serial-section analysis of stroke form and top structure. An attempt will be made to integrate this information into a unified theory of ciliary beat based on the interaction of various ciliary organelles.

Major Findings: Acetylcholine, calcium chloride, epinephrine, progesterone and prostaglandin E₁ and E₂ were not particularly effective in producing ciliary activity in culture since few reduced tips (8S + 2 or less) could be observed in thin-section studies and few groupings in coordinated activity by scanning electron microscopy. Estrogen and potassium chloride treatment in vitro produced increased ciliary activity demonstrable by both transmission and scanning electron microscopy. In the presence of serotonin or serotonin with ATP, reduced tips were extremely frequent in thin-section images, this being evidence of considerable ciliary activity. By scanning electron microscopy, ciliary bends were particularly deep, and cilia in any one area would point in different directions, as is common for actively beating cilia.

Preliminary scanning electron microscopic analysis of human oviducts has demonstrated that menopausal women retain considerable numbers of their ciliated cells, and that many of the cilia appear to be actively beating, both in the fimbria and ampullae.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the mechanism of action of oviductal cilia and their role in gamete transport.

Proposed Course: The major objectives of this program should be completed within this contract period.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural Studies on the Oviduct of the Intact Normal, the Ovariectomized and Ovariectomized-Hormone Treated Rabbit and Pig-Tailed Monkey, Macaca-Nemestrina
Contractor: Medical College of Virginia
Money Allocated: \$13,195 (FY 70), \$9,200 (FY 71), \$15,190 (FY 72)
\$18,760 (FY 73)

Objectives: This research project is part of an interdisciplinary research program on oviduct structure and function being carried out at the University of Washington. This investigator will carry out at the University of Virginia certain morphological aspects of the work on specimens obtained in Seattle where correlative in vivo observations and physiological measurements are being made on the same material.

A systematic study of the ultrastructure of the fimbria, ampullae, and isthmus of the normal rabbit oviduct in the pre- and post-ovulatory period, in the ovariectomized animal, and in ovariectomized animals receiving estrogens and progesterone singly and in combination will be made. Studies will not be confined to the epithelium but will include fine structural observations on the smooth muscle which may be correlated with changes in oviduct motility. With this base line established on rabbits, similar studies will be made on the oviduct of the pig-tailed macaque.

Major Findings: Observations on the fimbrial oviductal tissue of the 9 mid-cycle and 3 estradiol-treated pig-tailed monkeys have utilized 1 μ sections for light microscopy and thin sections for electron microscopy from at least 2 blocks of each animal, approximately 700 electron micrographs having been taken. In contrast to what was expected, deciliated cells in various stages of ciliogenesis were found in limited, but easily detectable, numbers in 3 of the mid-cycle and all 3 of the estradiol-treated animals. Unusual apical protrusions of ciliated cells containing variable numbers (2-28) of ciliary axonemal complexes (9+2) lay within the cytoplasm lacking their plasma membrane coverings. This is a possible mechanism of deciliation and was noted in 5 mid-cycle and 1 estradiol-treated monkeys. The secretory cells of the mid-cycle animals varied appreciably between different animals and to a lesser extent in the same animal and differed in some respects from those of the estradiol-treated animals. The variations included the degree of complexity of the apical cell borders; the number, density and size of secretory granules; and the number of GER profiles and free particles of glycogen.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the ultrastructure of the mammalian oviduct and its responses to changes in the hormonal environment.

Proposed Course: Since the major objectives of this contract will be met within this contract period, the contract will be discontinued.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Anti-luteinizing Activity of Follicular Ovum
Contractor: University of Illinois
Money Allocated: \$43,365 (FY 69), \$45,635 (FY 70), \$46,941 (FY 71)
\$28,173 (FY 72)

Objectives: Recent work from this laboratory has shown that removal of the egg and surrounding granulosa cells from a ripe ovarian follicle (rabbit) initiates luteinization and a short spurt in progesterone secretion. Hence the egg seems to exert an anti-luteinizing activity. A series of well-planned studies to elaborate and determine the significance to this observation are being carried out under the terms of this contract.

Major Findings: New experiments have failed to substantiate the presence of a luteolytic agent in porcine follicular fluid.

Significance to Biomedical Research and Program of the Institute: Reproductive function in the human appears to depend on corpus luteum luteinization and luteolysis which have thus been identified as particularly important topics to the purposes of the contraceptive development program.

Proposed Course: The program was discontinued in view of the negative findings.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Endocrine Control of Human Oocyte Maturation
Contractor: Peter Bent Brigham Hospital
Money Allocated: \$23,800 (FY 71) \$49,591 (FY 72), \$38,832 (FY 73)

Objectives: The objective of this contract is to explore a new approach to contraception involving the control of maturation of the human oocyte. Mammalian oocytes are in a resting stage of meiosis until shortly before ovulation. At this time meiosis resumes. The investigators wish to find out what hormones stimulate the resumption of meiosis in humans. They propose to administer these hormones before ovulation in order to induce a premature maturation of the oocyte. They anticipate that if this is done, when ovulation does occur the over-mature oocyte will be incapable of being fertilized.

Major Findings:

(1) In both the light and electron microscope studies the stage of the menstrual cycle and reproductive state of the patient did not appear to influence the size of the follicle obtained. Moreover, follicle size could not be correlated with the degree of maturation of the 44 oocytes studied by both techniques, since a wide range of follicle size was represented in all the oocyte categories described.

(2) Initial correlations tend to suggest that follicles containing oocytes with metaphase chromosomes or polar bodies have relatively lower concentrations of steroids. Extreme levels of steroids, high or low, in the antral fluid are usually associated with the presence of atretic oocytes.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraception Development Branch to support research on new approaches to contraception involving the control of human oocyte maturation.

Proposed Course; Discontinuance. Objectives have been met and further work will be conducted under grant funding.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Fertilization of Mouse Ova In Vitro by Epididymal Sperm
Contractor: The Jackson Laboratory
Bar Harbor, Maine
Money Allocated: \$58,535 (FY 72) for two years, \$31,000 (FY 74)

Objectives: The investigators have been highly successful in obtaining in vitro fertilization (95%) of mouse ova by epididymal sperm. In this contract proposal, it is proposed to define the chemical, physical, temporal, and genetic factors that have contributed to this success.

To the objective of obtaining fertilization in the shortest interval and with the minimum number of sperm, the investigators will study the effect of varying systematically, singly or in combination, ingredients of the medium and other factors of the in vitro system; among the important parameters to be studied are the necessity of subjecting epididymal sperm to capacitation, the effect of egg denudation, acrosome reaction, and patterns of attachment of the sperm head to the zona pellucida.

Major Findings:

- (1) Sperm from cauda epididymis have a much higher fertilizing ability than sperm from the corpus or caput.
- (2) The presence of oviductal or epididymal fluids is not required for successful in vitro fertilization.
- (3) In vitro fertilization takes place somewhat more rapidly than in vivo with comparable fertilization rates.
- (4) Aging of ova at 37°C for up to 12 hours in a culture medium prior to semination was without deleterious effect on fertilization. Longer aging decreased ovum viability. Storage of ova at 2°C retarded the negative effect of aging.
- (5) Bovine serum albumin (BSA) in concentration of at least 3 mg/ml of medium was a required component of the Whitten medium for the support of normal fertilization rate. Crystalline egg albumin at 10mg/ml of medium was considerably less effective than BSA.
- (6) Presence of cycloheximide was not compatible with fertilization even though it was without effect on sperm motility.

Significance to Biomedical Research and Programs of the Institute: The work to be carried out on this project is directly related to the published purpose of the Contraceptive Development Branch to support research aimed at clarifying factors responsible for successful fertilization.

Proposed Course: This contract will not be renewed because the objectives will be met at the end of the present contract year.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Morpho-Physiologic Studies on Reproductive Organs and Germinal Cells of Free Ranging Male and Female Macaques.
Contractor: Harbor General Hospital
Money Allocated: \$23,783 (FY 69), \$42,416 (FY 70), \$45,978 (FY 71), \$39,714 (FY 72), \$52,519 (FY 73)

Objectives: The main objective of the proposed research is to study in vitro fertilization of primate ova for the purpose of (1) defining the experimental conditions suitable for gamete interaction, and (2) investigating the morphologic changes associated with fertilization and early embryonal development. Two species of primates are to be used: the free-ranging rhesus macaque and the squirrel monkey (*Saimiri sciureus*).

Major Findings: (1) Meiotic maturation is accompanied by drastic changes of the oocyte mitochondria which, in meiotically maturing and mature oocytes, assume morphological characteristics typical of mitochondria of steroid secreting cells. This finding suggests that, during meiosis, oocytes may become engaged in synthesis and/or metabolism of steroid hormones which could be necessary for their maturation and/or incipient follicle luteinization. (2) Testicular biopsies obtained from adult rhesus males in the breeding and non-breeding season were studied by means of high resolution light microscopy and electron microscopy. It was possible to note that salient differences characterize the seminiferous tubules of these animals in the two periods. In the breeding season, the histology and cytology of the seminiferous tubules were normal, and germ cell differentiation and sperm production appeared to occur in a normal pattern. The walls of the seminiferous tubules were populated by germ cells in various stages of differentiation, from spermatogonia to spermatids and young spermatozoa. The Sertoli cells also showed normal architecture and normal morphological characteristics. In contrast, testicular tissue from non-breeding animals revealed universal atrophy and disorganization of the seminiferous tubules, and complete or nearly complete spermatogenic arrest. For most of their extension, the walls of the tubules consisted predominantly of Sertoli cells with only a few spermatogonia present, none of which was found to be in mitosis. The Sertoli cells exhibited massive amounts of gigantic lipid droplets filling most of the cytoplasm. (3) So far, it has been impossible to obtain fertilization of rhesus macaque oocytes in culture.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch as it pertains to the mechanisms involved in the critical process of fertilization.

Proposed Course: Discontinuation. Most of the objectives have been achieved.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Effects of Intrauterine and Embryonic Elevation on Pregnancy in Experimental Animals.

Contractor: Jefferson Medical College

Money Allocated: \$37,660 (FY 70), \$44,220 (FY 71), \$49,322 (FY 72), \$62,408 (FY 73)

Objectives: The investigator proposes to develop methods of administering microwave irradiation to the uteri of experimental animals, and to investigate the effects of the microwave energy on the embryos and surrounding tissues in the intact rat and rabbit at various stages of gestation.

Major Findings: Previous reports have dealt with the ability to cause embryonic resorptions in the rat using 2450 MHz microwave radiation. Both antenna directed and non-directed radiation has been utilized, and in certain situations exposure of the exteriorized rat embryonic sites resulted in complete resorption. In an effort to control the temperature of the uterus more accurately, thermocouples were utilized to measure uterine, skin and abdominal temperatures.

Antennae used to direct radiation to the embryos within the closed abdomen produced "hot spots" in the near field, so that intact pregnant animals developed areas on the body where excessive heat has been absorbed. This can be corrected by utilizing a rotating coaxial connector, so that the antennae can be rotated during the radiation. Results indicate that pregnancy can be completely interrupted in the intact rat without causing areas of excessive heating. This has been a most encouraging development and reinforces the propriety of pursuing the development of a 915 MHz microwave generator which is more penetrating.

Significance to Biomedical Research and Program of the Institute: The work undertaken on this project is important in understanding the interaction of pelvic tissues with radiation and related to the goal of the program aimed toward the development of new contraceptive technology.

Proposed Course: This contract has been discontinued since objectives have been achieved.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Progesterone and Uterine Secretion
Contractor: Case Western Reserve University
Money Allocated: \$99,680 (FY 73) for 2 years

Objectives: The purpose of this study is to examine the metabolism in the uterus of the rabbit of radioactively labelled progesterone and to ascertain whether or not reduction of progesterone to pregnane-3,20-dione (dihydroprogesterone) is a prerequisite to progestational activity in this tissue. This consideration is similar to the "5 α -reduction" of testosterone to dihydrotestosterone in the androgenic action of the male hormone.

The work to be conducted includes the study of the physiological fate of injected ³H-labelled progesterone, its metabolic clearance rate, the rate of extraction from the blood by the uterus, and the chemical changes which the hormone undergoes during these processes.

Major Findings: The early phases of this project required the development of techniques for measuring the capacity of the uterus to concentrate progesterone and extract it from the circulating blood. As reference, the tissues of the rabbit's head were used for comparison. A most unexpected finding was that the head (brain tissue, etc.) possessed a much greater capacity than the uterus for extracting labelled progesterone from the blood. The significance is not clear, but another anatomical element (a limb) will be used as reference for the uterine studies.

Another finding (which involved the development of a sensitive assay method for 5 α -dihydroprogesterone in plasma) was that very little, if any, of the latter reduction product of progesterone was reconverted to the parent substance progesterone. In other words, the formation of 5 α -dihydroprogesterone from progesterone under physiological conditions is an irreversible transformation.

Significance to Biomedical Research and Program of the Institute: The work undertaken, involving the mode of action of progesterone on the uterus, is related to the program of the Contraceptive Development Branch to support research in this aspect of reproductive physiology.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Steroid Hormone Receptors in Human Cervix
Contractor: The University of Texas Medical School at Houston
Money Allocated: \$71,981 (FY 73 for 2 years)

Objectives: The investigator proposes to: (1) establish the existence of specific cytoplasmic estradiol and progesterone receptors in human cervical tissue; (2) show that these receptors are functionally similar to those obtained from other target tissues; (3) study the number of cytoplasmic receptor sites and/or their degree of saturation as a function of cycle stage and hormone environment in individual patients.

Major Findings: It has been found that high speed (142,000 x g) supernatant fractions from extracts of human cervical tissue contain macromolecules which specifically bind ^3H -estradiol- 17β and are subject to degradation by proteolytic enzymes. Using dextran-coated charcoal to separate bound from free hormone, the association constant was shown to be 0.70 nM^{-1} at 0° and 0.74 nM^{-1} at 24° , pH 8.0. Relative affinity constants determined from the ability of unlabeled steroids to compete with ^3H -estradiol for binding sites were: estrone, 0.43; estriol, 0.37; dihydrotestosterone, 0.003; ~~5 α~~ -androstane- $3\alpha, 17\beta$ -diol, 0.001. Testosterone, cyproterone acetate, progesterone, corticosterone, and cortisol did not compete in >3000 fold molar excess, indicating that this protein is neither the sex steroid nor the cortisol binding globulin from serum. Estradiol binding activity measured as available sites was distributed throughout the anatomical regions of the cervix with the highest concentration associated with the columnar epithelium. The association constants in all regions were similar and comparable with that of the corresponding endometrium. The concentrations of binding sites from proliferative phase endometrium and cervix were significantly higher than from secretory phase samples when expressed per g but not per mg protein.

Significance to Biomedical Research and Program of the Institute: The characterization of hormone receptor complexes in human cervix is directly related to the published purpose of the Contraceptive Development Branch to support studies directed towards the development of new female contraceptives.

Proposed Course: This is a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Sex hormone Receptors in the Cervix and Cervical Response to Sex Hormones.

Contractor: The Albany Medical College

Money Allocated: \$82,400 (FY 74 - for 2 years)

Objectives: The investigator proposes to investigate some of the parameters of cervical biology that intervene between the arrival of plasma borne estrogen and progestin molecules at the boundary of cervical secretory cells and the response of those cells. The properties and the concentration of the cytoplasmic estrogen receptor and progesterone receptor proteins of human cervical tissue will be determined.

Significance to Biomedical Research and Program of the Institute: The characterization of hormone receptor complexes in human cervix is directly related to the published purpose of the Contraceptive Development Branch to support studies directed towards the development of new female contraceptives.

Proposed Course: This is a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT

July 1 , 1973 through June 30, 1974

Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Estrogen and Progesterone Receptors in the Cervix during
the Menstrual Cycle of the Chimpanzee

Contractor: Emory University

Money Allocated: \$108,782 (FY 74) for two years

Objectives: The main objective of this research program is to investigate the mechanisms through which changes in the circulating levels of ovarian hormones influence the physiology of the cervix. In order to accomplish this task the contractor will examine the characteristics of cervical hormone receptors during the menstrual cycle and attempt to relate them to the changes in cervical histology, cervical mucus properties and endogenous levels of ovarian hormones.

Significance to Biomedical Research and Program of the Institute: The understanding of factors which influence the physiochemical properties of cervical mucus which in turn influence sperm penetration is directly related to the objectives of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptive approaches and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Coordinated Approach to the Physicochemical
Characterization of Human Cervical Mucus
Contractor: University of Pennsylvania
Money Allocated: \$58,307 (FY 74)

Objectives: The investigator proposes to (1) study the role of pH, ionic strength, and protein concentration in determining the rheological properties of native and lyophilized cervical mucus from normally ovulating women, and infertile women; (2) interrelate rheological properties with routine biological tests employed in the clinical evaluation of the mucus and plasma levels of progesterone and estrogen; (3) establish standard conditions for the reconstitution and study of pooled, lyophilized cervical mucus fractions; and (4) evaluate the effect of various mucolytic agents on the macromolecular composition and rheological properties of cervical mucus, providing a molecular basis for mucin solubilization.

Significance to Biomedical Research and Program of the Institute: The work undertaken on this project is important in understanding the biophysical and biochemical character of cervical mucus during normal and abnormal menstrual cycles and is related to the published purpose of the Contraceptive Development Branch to support studies directed towards the development of new female contraceptives.

Proposed Course: This is a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Biochemical Characteristics of Cervical Mucus of
Macaca radiata (Bonnet Monkey) during Menstrual Cycle.
Contractor: Harvard Medical School
Money Allocated: \$194,012 (FY 74 for 3 years)

Objectives: The investigator proposes to ascertain the relationship between the chemical structure of the macromolecular components of mucus and its physical and physiological properties. The main macromolecular components responsible for the viscoelastic properties of the mucus is a glycoprotein, the structure of which will be completely elucidated. An attempt will be made to find changes of this structure during the menstrual cycle, in order to establish the biochemical mechanism responsible for these changes and ways of controlling it.

Significance to Biomedical Research and Program of the Institute: The work undertaken on this project will provide needed basic information about primate cervical mucus, its glycoproteins, and changes which may take place during the menstrual cycle and is related to the published purpose of the Contraceptive Development Branch to support studies directed towards the development of new female contraceptives.

Proposed Course: This is a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of an In vitro Model System for the Study of the Dynamics of Sperm Transport through the Human Cervical Canal.
Contractor: New York Medical College
Money Allocated: \$62,492 (FY 74)

Objectives: The aim of the project is to characterize and standardize a model system - the in vitro human uterus-cervix-vagina preparation - in order to determine the optimal in vitro conditions for the study of the dynamics of sperm transport through the human cervix. The ionic environments involved will be studied by the use of different types of perfusion solutions in order to find the perfusion medium which most closely resembles whole blood in terms of normal cervical function, such as secretion and motility. The role of perfusion additives other than ions, such as amino acids, glucose and lactate, and dextran, will be determined. The optimal rate of perfusion will be found in terms of normal motile function and maximal functional life in vitro. The physiology and pharmacology of this model system will be studied by infusion techniques as a method comparing its in vitro physiological behavior and pharmacological responses to the reported behavior and responses of the human female reproductive tract in situ.

Significance to Biomedical Research and Program of the Institute: The work undertaken on this project is important in understanding the dynamics of sperm transport through the cervix and into the uterus, and is related to the goals of the program aimed towards the development of new contraceptive technology.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NIHED ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Studies on Sperm Transport Through the Cervix
Contractor: University of Washington
Money Allocated: \$ 53,955 (FY 74) for two years

Objectives: The objectives of this research are to explore the mechanisms of sperm penetration through the cervical mucus both in vitro and in vivo and to explore whether sperm penetration can be influenced by a variety of chemical approaches.

Significance to Biomedical Research and Program of the Institute: Research dealing with sperm transport through the female reproductive tract is essential for the formulation of new methods of fertility regulation and is directly related to the mission of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of an In vitro Model System for the Study of the Dynamics of Sperm Transport through the Human Cervical Canal.

Contractor: New York Medical College

Money Allocated: \$62,492 (FY 74)

Objectives: The aim of the project is to characterize and standardize a model system - the in vitro human uterus-cervix-vagina preparation - in order to determine the optimal in vitro conditions for the study of the dynamics of sperm transport through the human cervix. The ionic environments involved will be studied by the use of different types of perfusion solutions in order to find the perfusion medium which most closely resembles whole blood in terms of normal cervical function, such as secretion and motility. The role of perfusion additives other than ions, such as amino acids, glucose and lactate, and dextran, will be determined. The optimal rate of perfusion will be found in terms of normal motile function and maximal functional life in vitro. The physiology and pharmacology of this model system will be studied by infusion techniques as a method comparing its in vitro physiological behavior and pharmacological responses to the reported behavior and responses of the human female reproductive tract in situ.

Significance to Biomedical Research and Program of the Institute: The work undertaken on this project is important in understanding the dynamics of sperm transport through the cervix and into the uterus, and is related to the goals of the program aimed towards the development of new contraceptive technology.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Studies on Sperm Transport Through the Cervix
Contractor: University of Washington
Money Allocated: \$ 53,955 (FY 74) for two years

Objectives: The objectives of this research are to explore the mechanisms of sperm penetration through the cervical mucus both in vitro and in vivo and to explore whether sperm penetration can be influenced by a variety of chemical approaches.

Significance to Biomedical Research and Program of the Institute: Research dealing with sperm transport through the female reproductive tract is essential for the formulation of new methods of fertility regulation and is directly related to the mission of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Sperm Antibody Activity in Cervical Mucus of Women
Contractor: New York Medical College
Money Allocated: \$199,475 (FY 74 for 3 years)

Objectives: It is proposed to study sperm agglutinating and sperm immobilizing antibodies and immunoglobulins in the cervical mucus of women with infertility problems and in control samples from normal women, and to improve the sperm antibody detection in these secretions. In all cases, the blood serum will be evaluated for the presence of agglutinating antibodies and correlated with the level of estradiol. Immobilization techniques and fluorescent antibody techniques will be further perfected and the agglutination methods adapted to a microscale. The preparation of soluble antigens from spermatozoa is planned in order to develop precipitation methods with their antigens as a means of improving sperm specific antibody detection in cervical mucus.

Significance to Biomedical Research and Program of the Institute: The improvement of sperm specific antibody detection in cervical mucus and the study of sperm antibodies and immunoglobulins in human cervical mucus is related to the goals of the program aimed towards the development of new contraceptive technology.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of a Laboratory Screening Procedure for Inhibitors of Progesterone Synthesis
Contractor: Harvard University
Money Allocated: \$45,120 (FY 70), \$45,500 (FY 71), \$83,567 (FY 72)
\$181,982 (FY 74 for two years)

Objectives: The objective of this contract is to define and evaluate agents which interfere with the enzymatic synthesis of progesterone in the corpus luteum. Specifically, the contract concerns the possibility of finding substances which are specific inhibitors of the mitochondrial system which converts cholesterol to pregnenolone; specifically, substances which act at the cytochrome P-450 site. It is suggested that substances exist which act specifically at this site and are non-toxic; such substances would be potential antifertility agents.

The immediate objective of this contract is to devise a convenient and relatively rapid screening procedure which will enable the prediction and preparation of substances which would be biologically effective for the termination of early pregnancy.

Major Findings: Work continued on the structure-activity relationships of inhibitors of cytochrome P-450^{cholesterol} in corpus luteum and placental mitochondria. Two types of inhibitors have been identified: those which interact with the apoprotein presumably at the site of substrate binding and those which interact with the heme prosthetic group. These two types are identifiable spectroscopically by characteristic spectra.

Three assay techniques have been developed and are being compared: a spectroscopic assay which is designed to identify the site of action and hopefully anticipate specificity, an in vitro enzymic assay which confirms inhibitory potency, and, third, an in vivo animal assay for luteolytic activity in the total animal. The latter test provides information on potency and toxicity in the presence of other biological systems. The first two assays yield comparable results. Comparison studies with the in vivo assay are not completed.

In the placenta, two cytochrome P-450s have been identified and partially separated. One is responsible for cholesterol side chain cleavage. This cytochrome responds almost identically to inhibitors as does the ovarian cytochrome P-450. The function of the other placental P-450 is not yet established.

Studies with cyanide-containing steroids indicate that some of these steroids are potent inhibitors of cytochrome oxidase of the conventional electron transport system. These are being studied further because of their potency and perhaps general toxicity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support development of laboratory screening procedures.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Study of Neural Regulation of Ovarian Function
Contractor: Southwest Foundation for Research and Education
Money Allocated: \$29,000 (FY 69), \$31,146 (FY 70), \$31,146 (FY 71)
\$64,652 (FY 72), \$76,951 (FY 74)

Objectives: The objective of the proposed study is to investigate the influence of the anti-inflammatory corticoid triamcinolone acetonide on the menstrual cycle of the baboon. The following end points will be explored: (1) dose-response relationship; (2) the effect on follicular development and on the endometrium; (3) the effect on luteinization when given immediately after ovulation, and (4) effect on the endogenous release of cortisol.

Major Findings: Injections of 2 mg. of triamcinolone on three consecutive cycles consistently delayed follicle development, but the menstrual cycle lengths were quite consistent and regular. Plasma level of LH was elevated in the early follicular phase and postovulatory LH surge was suppressed. Luteinizing follicle and hypotrophic endometrium was observed on histology.

A single injection of 4 mg of triamcinolone after ovulation elevated plasma level of LH, but length of luteal period was unaltered.

Daily injections of TRH(40 μ g) at the time of ovulation suppressed pre-ovulatory LH surge; however, postovulatory LH level was elevated.

Alteration of plasma level of LH by injection of triamcinolone or TRH may be due to change of the feedback action of circulating ovarian or adrenal hormones on hypothalamus-controlled pituitary function.

Significance to Biomedical Research and Program of the Institute: The study of compounds that can block ovulation is directly related to the published purposes of the Contraceptive Development Branch.

Purposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Pilot Study for the Large Scale Preparation of Purified Human Chorionic Gonadotropin
Contractor: Columbia University
Money Allocated: \$47,300 (FY 70), \$85,086 (FY 71), \$194,280 (FY 72 for two years), \$250,000 (FY 74 for two years)

Objectives: The objective of this investigation is to undertake a feasibility study to determine the optimal conditions for preparation of large batches of highly purified human chorionic gonadotropin (HCG). Secondary goals of this program are: (1) to obtain data bearing on the chemical and immunological characterization of HCG fractions which appear to be homogeneous in polypeptide content, but possess differing biological activities, and (2) to obtain data on the stability of HCG using both immunological and biological activities as guides.

Major Findings: Sizable quantities of purified hCG and its subunits have been made available for the NICHD distribution program.

Studies on the amino acid sequence of the terminal peptide of hCG- β indicates a dissimilarity of this material from hLH- β . Recombination of the essentially inactive subunits results in biological activity which is essentially indistinguishable from the native hormone.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support the development of procedures for human hormone isolation and purification.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: New Methods of Fertility Regulation: Specific Binding of Luteinizing Hormone to Cells of the Corpus Luteum and Inhibition of this Process.
Contractor: University of Texas - Houston, Texas
Money Allocated: \$119,340 (FY 69) for three years; \$84,187 (FY 72) \$19,754 (FY 73) \$147,282 (FY 74) for two years

Objectives: The objectives of this contract are twofold: (1) to complete the preparation and characterization of ovine luteinizing hormone subunits for distribution to other investigators via the Center for Population Research; (2) to prepare new derivatives of LH and its subunits and evaluate these derivatives using a radioligand assay.

Major Findings: The preparation and characterization of the ovine α - and β -subunits of LH (oLH α and oLH β) have been completed. Biological assays have been completed. Approximately 460 mg of each of the subunits are available for distribution and further investigation.

Guanidynylated oLH α + oLH β or guanidynylated oLH α + guanidynylated oLH β give potencies of less than 2% of LH by either the OAAD assay or the RLA assay. However, oLH α + guanidynylated oLH β had about 25% of the potency of LH by the OAAD assay or 55% as measured by the RLA assay. Thus if one retains a positive charge or a neutral charge on the amino functions of the beta subunit relatively good biological activity is retained.

With regard to the functional phenolic hydroxyl group studies, it has been concluded that the nitration of the tyrosine residues in the LH beta subunit does not significantly affect the ability of the two subunits to recombine nor does it materially affect the biological potency of the molecule as measured by its ability to combine with the receptor site in the radioligand assay; nevertheless, the activity as measured in the OAAD assay is significantly lower in this instance. When one nitrates more than one residue of tyrosine on the LH alpha subunit the potency of the molecule drops rapidly as studied by either the OAAD assay or the radioligand assay.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to elucidate the mechanism of action of the pituitary hormones.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of Radioimmunoassays for Rhesus Monkey
Gonadotropins
Contractor: Endocrine Laboratories of Madison, Inc.
Money Allocated: \$78,553 (FY 70), \$78,170 (FY 71), \$64,688 (FY 72)
\$97,190 (FY 73)

Objectives: This proposal is based on the recognition that rhesus monkey gonadotropic hormones do not cross-react immunologically with their human counterparts or at least not sufficiently to permit their detection in body fluids by radioimmunoassay. In this project, the investigators will collect urine from gonadectomized rhesus monkeys for the isolation of monkey LH and FSH. The urinary gonadotropins will be utilized because of the limited availability of monkey pituitary glands. The gonadotropins thus obtained will be used as antigens to obtain antisera for development of radioimmunoassays. Purification of the hormones will be by procedures previously developed, and by adaptations of methods developed in other laboratories and reported in the literature.

Major Findings: The contractor has optimized the procedures for the recovery of FSH from monkey urines. A heterologous RIA for monkey urinary FSH has been developed.

Starting with the crude acetone precipitate, NICHD researchers have purified the material 100 fold. Development of a homologous RIA is in progress.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support the development of new methodologies for the assay of steroid and protein hormones in the blood of primates including man.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of an Automated Immunoradiometric Assay System
Contractor: Veterans Administration Hospital
Palo Alto, California
Money Allocated: \$59,110 (FY 71), \$33,335 (FY 72), \$41,860 (FY 74)

Objectives: The objective of this contract is to establish a simple automated immunoradiometric assay device, capable of measuring low concentrations of a wide variety of antigens. The assay device is made possible by the prior development (by the applicant) of a new type of immunological assay system (the immunoradiometric assay). In this method, purified radioactive antibodies are used as reagents to convert all the unknown antigen into a directly detectable complex. Unreacted antibody is removed by reaction with an insoluble antigen immunoabsorbent. An important but parallel part of the project is the investigation and development of alternative antibody labels.

Major Findings: A prototype of an automated immunoradiometric assay machine has been constructed and tested. Assay precision is similar to previous manual 2-site IRMA assay systems but the "blank" (zero dose) radioactivity is significantly higher. Further experiments showed that Brij 58 over the concentration range 0.001 to 0.1 gm %, progressively reduced the "blank", but there was also significant suppression of the dose-response curve. KI and dithioerythritol were ineffective. Ascorbic acid reduced the "blank" but almost completely inhibited the dose-response. Repurification of the labelled antibody through Sephadex G 100, with or without preliminary treatment of the labelled antibody with charcoal, resulted in a very low "blank" and an improved standard curve.

2 species, 2-site IRMA systems using ^{125}I -anti(IgG) have been further explored and characterized using "mirror image" systems - ^{125}I -GP.anti(R.IgG) and ^{125}I -R.anti(GP.IgG).

Continuous blood sampling has been established, preparatory to studying continuous monitoring of hormones in the blood.

Automatic data processing methods have been improved by the use of a weighted 4 parameter logistic curve-fitting assay program.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the development of new types of immunological assay systems.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Control of Ovulation in the Monkey
Contractor: Oregon Primate Center
Money Allocated: \$44,000 (FY 69) for two years \$47,543 (FY 71)
\$70,612 (FY 72)

Objectives: These studies are directed towards establishing the feedback site of progestins as inhibitors of ovulation in the rhesus monkey using experimental approaches previously used in the rabbit. These approaches are feasible in the rhesus monkey but much base-line information is being obtained preliminary to critical studies of feedback inhibition of ovulation.

Major Findings: During a 4-hour incubation of monolayer culture cells from the anterior pituitaries of cynomolgus monkeys, there was a significant release of LH into the media in response to synthetic LRF. The amount of LH released was directly related to the dose of LRF and the responsiveness to LRF was maintained for at least 10 days. The addition of estrogen with LRF suggested a potentiation of the LH released.

Compounds that stimulate levels of intracellular cyclic AMP were also tested in this monolayer culture system. Neither theophylline nor dibutyryl cyclic AMP stimulated LH secretion into the culture media above control levels whereas monkey hypothalamic extract or 10^{-8} M LRF led to an 8- and 6-fold stimulation respectively over control levels.

Significance to Biomedical Research and Program of the Institute: Studies of the mechanism of ovulation are clearly relevant to the purposes of the contraceptive development program.

Proposed Course: Discontinuance. Relevant research is being supported by a NICHD grant.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Endocrine Regulation of the Corpus Luteum
Contractor: Colorado State University
Money Allocated: \$26,706 (FY 69), \$37,220 (FY 70), \$53,618 (FY 71),
\$89,632 (FY 72), \$73,002 (FY 74)

Objectives: The primary objective of this research project is to elucidate the endocrine regulation of the ovine estrous cycle by measuring peripheral serum levels of FSH, LH, prolactin, estradiol, and progesterone. Radio-immunoassay methods are being developed for all these measurements. For the steroids, the methods are validated by comparison with double isotope derivative methods. Methods are also being developed to measure uptake of progesterone-C¹⁴ and LH-I¹²⁵ by uterus and ovaries respectively. Preimplantation embryos will be studied to determine if they have the ability to synthesize LH in vitro, and to metabolize six steroids in vitro.

Major Findings:

1. Administration of anti-LH and anti-prolactin sera to pregnant and nonpregnant ewes did not influence serum progesterone levels.
2. Immunization of sheep with ovine LH or prolactin was without effect on cyclicity or pregnancy in these animals, even though titers of circulating antibodies could be demonstrated at all times.
3. Metabolic clearance rate of gonadotropins is not influenced by the stage of the estrous cycle.
4. In monkeys, the high endogenous levels of progesterone during the luteal phase are not responsible for the low levels of LH and FSH during this phase of the cycle.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to describe quantitatively the hormonal components of the estrous cycle in relevant animal models.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Control of Ovulation and Corpus Luteum Function
Contractor: Medical College of Georgia
Money Allocated: \$43,760 (FY 70), \$25,891 (FY 71), \$41,836 (FY 72),
\$4,408 (FY 74)

Objectives: The investigators propose studies to gain insight into the action of estrogens at the hypothalamic and pituitary level. More specific studies will deal with steroid-protein interaction in the rat hypothalamus and pituitary and in the human pituitary. This work will involve the use of both radioactive estradiol and testosterone and the possible identification of the testosterone metabolite which binds to the cytosol fraction. This study will include the use of hormone analogs that bind covalently to the 8S-receptor.

Major Findings: Investigation of the interactions between estradiol and specific cytosol receptor molecules in the anterior pituitary and hypothalamus of the rat has been performed and has yielded the following results: (1) estradiol-receptor complexes can be identified and semi-quantified by gel filtration, equilibrium dialysis and sucrose gradient centrifugation; (2) no specific binding of testosterone or 5 α -dihydrotestosterone is demonstrable; (3) Scatchard plot analysis shows that the extent and affinity of estradiol binding is independent of sex or the presence of gonads, and that one species of receptor can be detected; (4) concentration of receptor sites in the pituitary is approximately equal to that found in the uterus and ten times that found in the hypothalamus; (5) invariant kinetic constants for association and dissociation rates in either tissue from both sexes suggest that the receptor molecules and the nature of the interactions are the same. It appears that sex differences in receptor content cannot account for the differences between cyclic gonadotropin secretion observed in the female and the relatively constant secretion in the male.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the action of the pituitary gonadotropins in the reduction of ovulation and the control of corpus luteum formation, function, and regression.

Proposed Course: Discontinuance. Original objectives were met.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Regulation of the Changes in Corpus Luteum Physiology
Required for the Establishment of Pregnancy in the Rat
Contractor: Case Western Reserve University
Money Allocated: \$53,313 (FY 70), \$53,313 (FY 71), \$45,812 (FY 72)
\$50,985 (FY 74)

Objectives: The proposed experiments are designed to shed further light upon the interaction between pituitary LH and prolactin and a placental luteotrophic hormone in the regulation of the rat corpus luteum. Interaction between these agents is assumed to be responsible for differences between the corpus luteum of pseudopregnancy and the corpus luteum of pregnancy, the latter being characterized by (a) increased size, (b) ability to maintain increased peripheral plasma levels of progesterone and (c) ultimate development of independence, i.e., the ability to function in the absence of continued support from the pituitary placenta. The fundamental hypothesis to be tested is that in the nonpregnant state LH has a luteolytic action, and the addition to the system of the placental luteotrophic hormone converts this action to a synergistically luteotrophic action.

Experiments will be carried out in the pregnant rat on three principal problems: (1) increase in size of the corpus luteum of pregnancy, (2) elevation in plasma progesterone levels, and (3) development of corpus luteum independence in the absence of pituitary and placenta. Methods to be used include various surgical manipulations, progesterone determination by competitive binding assays, and prolactin determination by radioimmunoassay.

Major Findings: Ergocornine treatment of pseudopregnant rats and of pseudopregnant rats bearing decidual tissue produced different results. Rats with decidual tissue were much more resistant to the progesterone suppressing effects of ergocornine. The data suggest that decidual tissue produces a luteotropic hormone which can sustain progesterone secretion in the relative and temporary absence of prolactin.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on corpus luteum physiology.

Proposed Course: Discontinuance in favor of NICHD grant. Initial contract objectives are to be met during the current contract period.

NICHD ANNUAL REPORT

July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Simultaneous Theoretical and Empirical Approach
to the Study of the Rat Estrous Cycle
Contractor: Northwestern University
Money Allocated: \$83,412 (FY 73)

Objectives: The investigator plans a systems analysis approach to the understanding of the mechanisms regulating reproductive cyclicality and acyclicity in the rat. The total approach includes the following stages to be carried out simultaneously: identify from existent experimental data the essential variables and connecting linkages among system components; make a model of the system; simulate the system by computer; perform "experiments" using the model and simulating by computer to compare the model to similar experiments in the real system; use the computer to simulate the results of new experiments; perform these experiments and modify the model if necessary, etc.

Because data on few of the variables needed for constructing an adequate simulation are available from the literature, the investigator will measure the following parameters and variables using radioimmunoassay and competitive protein binding methods for steroids and gonadotrophic hormones: (1) estrogen progesterone, LH, FSH and prolactin in serum throughout the four-day cycle; (2) distribution volumes and loss rate constants of the above hormones in cyclic rats and following ovariectomy and/or hypophysectomy; (3) setpoints for LH, FSH and prolactin in prepubertal and adult rats; (4) the negative feedback constant for the suppression of LH and FSH secretion by estrogen; (5) ovarian transfer functions, determining the effects of gonadotropins on estrogen and progesterone secretion and on morphology; (6) estrogen, LH, and FSH levels during the onset, steady state and termination of persistent estrus due to continuous light.

Major Findings:

- (1) The computer simulation model of the estrous cycle which employed a linear equation for calculation of LH secretion rate is incorrect.
- (2) The relationship between the intrauterine fluid content and hormone levels was explored. The circulating level of progesterone does not appear to be the determinant for fluid release. LH causes the disappearance of intraluminal fluid consistently at a lower dose than it causes ovulation; the dose ratio for the same two variables is closer in the FSH treated rats.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the mechanisms regulating reproductive cyclicality and acyclicity.

Proposed Course: Termination in favor of NICHD Research Grant.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Effect of Prostaglandins on Ovarian Function
Contractor: University of Miami
Money Allocated: \$115,909 (FY 71) for two years

Objectives: The objective of this research is to study the in vivo effect of prostaglandin $F_{2\alpha}$ on the corpus luteum of the menstrual cycle in women in order to determine what acute effects this compound has on the corpus luteum and to determine if it can be used as a luteolytic agent in humans. The volunteer subjects will be divided into two groups. The first group of normal healthy ovulating non-pregnant females will receive an infusion of prostaglandin $F_{2\alpha}$ at different times in their secretory phase of the cycle. This will be considered the control group. The parameters for corpus luteum function will be the measurement of plasma progesterone in the peripheral venous blood. The second group will consist of patients already scheduled to undergo abdominal gynecologic surgery for non-endocrine disease. Their surgery will be scheduled at a time a corpus luteum of a certain age can be expected. Prostaglandin $F_{2\alpha}$ administration will begin at varied times before surgery and progesterone will be measured in peripheral venous blood and in blood obtained from both ovarian veins. In addition, steroidogenesis of incubated luteal tissue will be measured in vitro.

Major Findings: Preovulatory infusion of $PGF_{2\alpha}$ in the monkey had no influence on the menstrual cycle length. In the human female infusion of $PGF_{2\alpha}$ has an equivocal effect on cycle length when it is done during the follicular phase and little or no effect when it is done during the luteal phase.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the effects of prostaglandins on the corpus luteum.

Proposed Course: Discontinue. Prostaglandin effect on the human CL could not be demonstrated.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Isolation of a Bovine Uterine Luteolytic Factor
Contractor: Cornell University
Money Allocated: \$107,232 (FY 69) for three years, \$53,789 (FY 72)
\$47,930 (FY 74)

Objectives: The principal investigator has prepared a bovine endometrial extract with the ability to regress corpora lutea of pseudopregnant, hysterectomized hamsters. The active material precipitates with ammonium sulfate and apparently is a large molecular weight protein; the possibility is being investigated that it may be prostaglandin $F_{2\alpha}$ adsorbed to a protein. These studies involve further fractionation and purification of the active material using preparative disc electrophoresis, and study of its effects on corpus luteum progesterone synthesis stimulated by LH, the effects of various modes of administration, and effects on ovarian blood flow and progesterone concentration.

Major Findings:

1. It is estimated that 1.0 mg/per g dried tissue, or more of arachidonic acid is present in the bovine endometrium during the estrous cycle. $PGF_{2\alpha}$ levels in the uterine venous blood increase greatly at the time of CL regression.
2. Infusion of arachidonic acid directly into corpora lutea resulted in a decrease in progesterone secretion, an increase in $PGF_{2\alpha}$ concentration in the ovarian venous blood and a concomitant increase in estrone concentration.
3. The biological activity of arachidonic acid is highly specific. Other compounds with similar degree of unsaturation were biologically inactive.

Significance to Biomedical Research and Program of the Institute: Corpus luteum regression, or "luteolysis" has been identified as particularly important to contraceptive development; therefore, this study is directly relevant to the purposes of the program.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Relationships of Endometrial Prostaglandins and Luteal Function
Contractor: West Virginia University
Money Allocated: \$35,337 (FY 69) for two years, \$32,460 (FY 71), \$46,086 (FY 72), \$43,204 (FY 74)

Objectives: The investigators are extracting the endometrium of sheep at various phases of the cycle to determine prostaglandins both qualitatively and quantitatively. The effects of progesterone on the corpus luteum and on prostaglandin levels and the effects of prostaglandin F_{2α} on corpus luteum function in hysterectomized animals are being studied. Corpus luteum function is being evaluated by progesterone levels and length of estrous cycle.

Major Findings:

- (1) Levels of PGF in endometrium and uterine venous plasma vary with day but not with status in pregnant and non-pregnant ewes on days 11-16 postestrus in ewes. Neither differences in transfer of PGF from uterine vein to ovarian artery nor differences in ovarian uptake of PGF appear to explain failure of high PGF to cause luteal regression in pregnant ewes.
- (2) Both cotyledonary and intercotyledonary endometrium contain considerable PGF in ewes and both tissues show increases in response to an IUD.
- (3) Injection of PGF_{2α} into the largest follicle on the ovary containing the corpus luteum induced luteal regression in 4 of 6 ewes at 200μg in 20 microliters. PGF_{1α}, PGE₂ and arachidonic acid were not effective at this dose. This system will be used as a tool to compare sensitivity of corpora lutea in pregnant and non-pregnant ewes to PGF_{2α}.
- (4) Uterine secretion of PGF in monkeys does not appear to correlate with luteal regression. Ovarian secretion of PGF_{2α} is being studied and data will be examined to see if this variable correlates with luteal tissue levels of PGF and estrogen and ovarian venous levels of estrogen.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to elucidate the factors responsible for the control of corpus luteum function.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Molecular Biology of the Human Corpus Luteum
Contractor: New York Medical College
Money Allocated: \$75,668 (FY 72); \$54,739 (FY 74)

Objectives: To study the biochemical aspects of the ageing or luteolysis of the human corpus luteum (CL) and to determine what biochemical changes occur when the CL's "life span" is prolonged by pregnancy. The studies which are conducted in vitro consist of the simultaneous measurement of the synthesis of progesterone and estrogen, the synthesis of de novo protein, RNA and DNA; the response of these separate biochemical processes to added gonadotropin (hCG) will also be measured. The major objective is to determine the nature of the luteolytic process, and the way in which it is prevented or reversed when pregnancy occurs.

Major Findings: The conditions for acquiring human tissues from surgery have been established. Optimum conditions have been determined for incubating the tissues, as well as the chemical procedures for measuring the various biochemical components from the same experimental specimens. A total of 32 human CL have been studied to date and the various measurements related to the age of the corpus luteum. This latter is established by conventional endometrial dating.

The major findings are that considerable variability exists in individual CLs of approximately the same age. This apparently reflects substantial variation in size and activity of CLs from different human subjects. The number of specimens examined so far provide no clear picture of any major trends in the biochemical parameters because of the high variability among human subjects. A larger series of specimens will have to be examined for any clear trend to become discernible.

At a basic level, there appears to be little synthesis in vitro of DNA. RNA and protein synthesis in vitro occurs, although the RNA and protein initially present in the tissue undergoes breakdown during the 30-minute incubation. Interpretation of this turnover in relation to age and to the concomitant synthesis of progesterone and estrogen will only be possible with a larger number of specimens.

Significance to Biomedical Research and Program of the Institute: Research on the human corpus luteum is essential in the understanding of the reproductive process and is directly related to the objectives of the Contraceptive Development Branch program.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new fertility regulating methods and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: New Methods for Fertility Regulation
Contractor: The Upjohn Company
Money Allocated: \$127,684 (FY 69) for two years, \$73,425 (FY 71)
\$88,140 (FY 72) for two years

Objectives: This is a study of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in which the conditions under which $PGF_{2\alpha}$ causes luteolysis and induces the termination of pregnancy will be determined, and the mechanisms by which $PGF_{2\alpha}$ achieves its luteolytic effect will be identified. Pregnancy termination is being studied in rabbits, guinea pigs, and rhesus monkeys by ovarian histology and ovarian and peripheral plasma progesterone levels. The period of pregnancy subject to interruption by $PGF_{2\alpha}$ -induced luteolysis is being established.

The second aspect of the proposal is an attempt to study the processes involved in the luteolytic effects of $PGF_{2\alpha}$. This includes studies of effects on gonadotropin activity, competitive inhibition of gonadotropins, direct effects on progesterone synthesis in the ovary by measurement of steroid levels in ovarian venous blood, and of the effects of prostaglandins on steroidogenesis by rat ovaries and primate corpora lutea in vitro. This also includes studies of cholesterol incorporation into progesterone, levels of adenyl cyclase, inhibition of effects of puromycin and cycloheximide, and potentiation of effects by phosphodiesterase inhibitors.

Major Findings:

- (1) Both the endometrium and the myometrium of human uteri show intake of PGE_1 and $PGF_{2\alpha}$. Monkey endometrium did not exhibit specific uptake of PGE_1 .
- (2) Prostaglandin binding in the rabbit oviduct has been demonstrated. Uptake of both PGE_1 and $F_{2\alpha}$ is greater in the ampulla than in the other segments of the oviduct.
- (3) The hamster myometrium has a specific binding-protein for PGE_1 which is associated with a membrane fraction of the cell and is dependent on the cell structure for binding specificity. The binding-protein is stabilized by association with its ligand PGE_1 .
- (4) In vivo treatment of ovariectomized hamsters with ovarian steroids showed that $PGF_{2\alpha}$ specific binding was greatly increased by progesterone and inhibited by estradiol. PGE_1 specific binding was increased slightly by progesterone and inhibited by estradiol.

Significance to Biomedical Research and Program of the Institute: The relevance of this study to the contract program is direct, particularly insofar as some of the work is being done in primates.

Proposed Course: This contract will be discontinued in FY 74 because the stated objectives will be fulfilled.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Prostaglandin: Its Role and Effect in Contraception
Contractor: University of California - Los Angeles
Money Allocated: \$63,767 (FY 72) for two years

Objectives: The objective of this research is to study the role of prostaglandins on calcium uptake and release by the sarcoplasmic reticulum and mitochondria from bovine and human uteri. The thesis is that the action of prostaglandins on uterine smooth muscle is due to the ability of these substances to regulate the intracellular stores of calcium. The primary aim of this research is to help to explain the mechanism of action of prostaglandins on the myometrium. Myometria from pregnant and nonpregnant cows will be fractionated to yield the sarcoplasmic reticulum and the mitochondrial fractions. Calcium transport in these fractions will be examined in the presence of various concentrations of prostaglandins. The proposed studies will be concerned with: (1) the effects of prostaglandins on calcium binding of bovine myometrial sarcoplasmic reticulum (SR) from nonpregnant animals at varying prostaglandin concentrations; (2) the role of progesterone in the sensitivity of uterine SR calcium transport to prostaglandins; (3) the interaction of prostaglandins with the SR membrane; (4) effects of prostaglandins on human myometrium as available; (5) the effects of prostaglandins on mitochondrial calcium binding in pregnant and nonpregnant animals.

Major Findings:

1. PGE₂ and PGF_{2 α} decrease the ATP-dependent calcium binding in SR isolated from nonpregnant cow myometrium. PGF_{2 α} also promotes release of bound Ca from SR. PGF_{1 β} , a physiologically inactive compound, has no effect on Ca binding or release.
2. The effect of the PGE₂ on binding is greater than that of PGF_{2 α} . This result provides the rationale for the clinical effectiveness of E₂ over F_{2 α} .
3. Progesterone increases the ATP-dependent Ca binding and antagonizes the effect of the PGs. This may mean that progesterone decreases the sensitivity of the uterus to PGs, a finding relevant to the determination of the effective dose of PG in contraception.
4. Oxytocin and PGs are synergistic in their action on ATP-dependent Ca binding and thus oxytocin increases uterine sensitivity to PGs.
5. The effect of PGs on Ca binding is not due to the ability of the PGs to complex ionic calcium since the action of PG on binding occurs at very low dose levels.

Significance to Biomedical Research and Program of the Institute: The study of the mechanism of action of prostaglandins is directly related to the stated interests of CDB in this group of substances.

Proposed Course: Discontinuance. Part of this study is being supported by a grant.

NICHD ANNUAL REPORT

July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Quantification of Prostaglandins
Contract: The Upjohn Company
Money Allocated: \$42,913 (FY 71) \$50,241 (FY 72)

Objectives: The objective of this contract is to develop a radioimmunoassay to measure prostaglandins and certain of their biologically active analogs, and subsequently to quantify these compounds in some body fluids and tissues.

Initially, prostaglandin E_2 and $F_{2\alpha}$ will be conjugated to bovine serum albumin and polylysine, then injected as a suspension with Freund's complete adjuvant into sheep, rabbits and guinea pigs to produce antibodies to the prostaglandin molecules. The affinity and specificity of the antibody will be characterized by comparing the competitive replacement of radiolabeled prostaglandin by several structurally related prostaglandin analogs. Conditions of incubation, methods of separation, and other alterable variables will be examined to optimize the sensitivity and precision of the assay. In addition, a limited number of prostaglandin analogs with biological activity that indicates a potential clinical advantage over natural compounds will be utilized similarly for assay development.

Circulating levels of prostaglandins will be quantitated during parturition in humans and at various stages of the menstrual cycle in monkeys. Absorption of prostaglandin from various formulations will be followed by quantitating prostaglandin in peripheral serum by radioimmunoassay. In vivo biological effects of administering antisera to prostaglandins will be determined.

Major Findings: Antibodies were produced, and assays developed to measure $PGF_{2\alpha}$, E_2 , the 15 keto and 13,14 dihydro-15 keto metabolites of $F_{2\alpha}$, 15(S) 15-me $F_{2\alpha}$ and 17 phenyl $F_{2\alpha}$. These assays have proven useful in measuring endogenous levels and following release from various pharmacologic preparations. In general, peripheral circulating levels of prostaglandin are not now thought to reflect importance in physiological terms. More important, probably, is the ability of a tissue to synthesize PG, and the factors controlling this.

Prostaglandin release was correlated with activity, in that blood levels of drug showed a variation closely associated with other biological endpoints measured.

Antisera developed are furnished to interested investigators, and should prove useful in elucidating the physiologic function of prostaglandins, as well as augmenting their pharmacologic potential. Antiserum to $PGF_{2\alpha}$ was without effect on pseudopregnancy in the rat or menstrual cycle in the rhesus monkey.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on radioimmunoassays of prostaglandins.

Proposed Course: Discontinued. Objectives have been fulfilled.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Role of Prostaglandins in the Secretion of
Gonadotropins
Contractor: Yale University
Money Allocated: \$61,445 (FY 73)

Objectives: The primary objectives of this research project are (1) to examine the possible role(s) of prostaglandins in the mechanisms controlling the pituitary secretion of LH and FSH, and (2) to study the effect of prostaglandin synthetase inhibitors and prostaglandin antagonists on the LH and FSH release induced by synthetic luteinizing hormone-releasing factor (LRF) in vitro.

Major Findings: Final results indicate that one or more of the prostaglandins may play an important role in the mechanisms regulating LH secretion from the anterior pituitary gland. This view is supported by the following findings: (1) PGE₁, PGE₂ and PGF_{2α} can act directly at the pituitary to increase LH release, (2) the pituitary responses to LRF are suppressed by the prostaglandin synthesis inhibitors, as well as by the prostaglandin antagonist, and (3) the indomethacin-induced suppression of the pituitary responsiveness to LRF can be reversed, as well as prevented, by PGE₂. Hence, prostaglandins appear to play a facilitory role in the action of LRF on LH release. The findings that prostaglandins increase pituitary adenyl cyclase activity and cyclic AMP concentrations in vitro suggest that the stimulatory effect of prostaglandins on LH release demonstrated in this study may be mediated by the adenyl cyclase-cyclic AMP system.

The results of this study further suggest that the regulation of LH release depends upon a complex and coordinated interrelationship among LRF, prostaglandins, and cyclic AMP. Synthetic LRF and hypothalamic extract stimulate adenyl cyclase activity and increase cyclic AMP concentration in rat pituitaries in vitro. Dibutyryl cyclic AMP and theophylline, even at low concentrations, were found to stimulate LH release. These results support the concept that the action of LRF on LH release is mediated by the adenyl cyclase-cyclic AMP system. This is consistent with the observation that dibutyryl cyclic AMP and theophylline augment the pituitary response to LRF. That LRF may play a role in regulating pituitary prostaglandin concentrations is suggested by the demonstration of a marked increase in pituitary PGE and PGF concentrations as well as PGE release following treatment with LRF. Further, dibutyryl cyclic AMP and theophylline also increase pituitary PGE and PGF levels suggesting that the LRF-induced increase in pituitary prostaglandin concentrations may be mediated, at least in part, by cyclic AMP. The finding that dibutyryl cyclic and theophylline can reverse, as well as prevent, the indomethacin-induced suppression of the pituitary responses to LRF supports the view that prostaglandins may act, in part, at a site prior to the production of cyclic AMP to mediate and/or modulate the pituitary responsiveness to LRF. The specific role(s) of the prostaglandins in the mechanisms regulating LH release, however, remains unknown.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the effects of prostaglandins on ovarian physiology.

Proposed Course: Discontinued. The original contract objectives were met.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Role of Prostaglandins in Ovarian Physiology
Contractor: Yale University
Money Allocated: \$25,470 (FY 71), \$15,270 (FY 72), \$78,269 (FY 73)

Objectives: The objective of this research is to study the effects of prostaglandins on ovarian physiology in the rhesus monkey. Specifically, this entails a study of (1) the effects of prostaglandins on steroidogenesis in the developing follicle and corpus luteum; (2) the effects of prostaglandins on development and morphology of the follicle and corpus luteum, and the effects on ovulation; (3) the effects of prostaglandin antibodies and antagonists on ovarian function.

Major Findings: Depressing effect of DES and $\text{PGF}_{2\alpha}$ on monkey luteal function could not unequivocally be established.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the effects of prostaglandins on ovarian physiology.

Proposed Course: Discontinued. Equivocal data do not merit further support at this time.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Enzymes of the Sperm Acrosomes, Occurrence of the Inhibitors of These Enzymes in Seminal Plasma and the Role of the Enzymes and Inhibitors in Capacitation of Sperm and in the Penetration and Fertilization of Ova

Contractor: University of Georgia

Money Allocated: \$29,494 (FY 70), \$45,835 (FY 71), \$44,462 (FY 72)
\$69,986 (FY 73), \$162,429 (FY 74) for two years

Objectives: The objectives of this research project are: (1) to isolate and purify the five acrosomal enzymes from mammalian sperm; (2) to demonstrate their role in fertilization; (3) to elucidate the role played by enzyme inhibitors in seminal plasma (including decapacitation factor, DF); (4) to attempt to correlate DF activity with the inhibition of new sperm neuraminidase; and (5) to study uterine enzymes and their possible role in capacitation.

Major Findings :

(1) Affinity chromatography employing a variety of acrosin substrates has been only partially successful. Inhibitor affinity chromatography employing p-aminobenzamidine linked to Sepharose ϵ -aminocaproic acid with carbodiimides has been found to be effective affinity column for acrosin. The enzyme binds at pH values at or near 8 and is eluted at values of pH 4.5 or lower.

Affinity columns employing proflavin as the ligand gave good results for the separation of acrosomal proteases from other acrosomal enzymes.

Gel filtration chromatography has resulted in a 3-fold purification of acrosin as expressed in the ability to hydrolyze BAEE.

(2) Methods for the sequential release of acrosomal enzymes have been perfected. The data suggest that acrosin, pH-3-labile azocoll proteinase, hyaluronidase and arylsulphatase are located within the acrosomal space whereas some azocoll proteinase and neuraminidase are localized at the inner acrosomal membrane (IAM) or at the equatorial segment.

(3) Electron microscopic observations of spermatozoa subjected to the sequential treatment reveal that $MgCl_2$ treatment removes only the plasma and the outer acrosomal membrane. The subsequent detergent treatment removes IAM and the electron dense material from the equatorial segment.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the role of sperm acrosomal enzymes and their inhibitors in capacitation and fertilization. ✓

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Biochemical Requirements for Fertilization of Mammalian Ova
Contractor: University of Georgia
Money Allocated: \$34,122 (FY 69), \$45,351 (FY 70), \$46,519, (FY 71)
\$42,999 (FY 72), \$64,418 (FY 74)

Objectives: This research project involves the study of at least three enzymes present in sperm, the corona-removing enzyme (CRE) and two trypsin-like acrosomal enzymes which, the applicant hypothesizes, are involved in the penetration and removal of the corona radiata, the zona pellucida and the vitelline membrane. The applicant further postulates that "decapacitation factor"(DF) is a low molecular weight material which inhibits the CRE and thus blocks fertilization or capacitation. Both rabbit and human materials are used to study these points. Included as a related objective is the development of an effective medium and the proper physical conditions for in vivo fertilization and cleavage of human ova obtained from ovarian tissue removed at local hospitals.

Major Findings:

(1) Two pure peptides have been isolated from the pronase-digested ultracentrifuged sediment of bull seminal plasma which originally contained the bulk of decapacitation activity (DF). One is a basic peptide with an electrophoretic mobility at pH 6.5 slightly less than that of lysine. This peptide has an N-terminal lysine, contains no arginine, histidine, tryptophane, or tyrosine and is active in preventing fertilization by capacitated sperm at less than $20\mu\text{g}/10^5$ sperm. When first tested buffer salts were present. Analyses and calculations reveal that only $0.2\ \mu\text{g}$ of peptide was present per 10^5 sperm. Thus the DF basic peptide is the most potent decapacitating substance found so far being 25 times more potent than DF neutral peptide. The other peptide is electrophoretically neutral and has an N-terminal aspartic acid or asparagine. The neutral peptide has the following amino acid composition: Asp (4), Lys (2), Ser (3), Gly(3), tThr (1), Glu (1), Ala (0.6). This peptide was effective in inhibiting the fertility of capacitated sperm at about $5\mu\text{g}/10^5$ sperm. The DF activity of the peptides in partially purified form was destroyed by trypsin, pronase, carboxypeptidase A and B and DFP treated carboxypeptidase A. It was not destroyed by chymotrypsin or carboxypeptidase B. The DF activity in a peptide mixture was destroyed by mild acid hydrolysis, but not by cation re resin hydrolysis. Incubation at pH 8.0 and 13.0 had no effect on DF activity.

(2) The DF peptides and the DF protein inhibit the corona penetrating enzyme.

(3) A variety of naturally occurring agents and synthetic acrosin inhibitors inhibit fertilization when they are added as a vaginal contraceptive ingredient.

(4) Techniques have been established to demonstrate antifertility activity of seminal components in the in vitro fertilization assay.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to elucidate the mechanisms involved in sperm capacitation and in the critical process of fertilization.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Study on Human Acrosomal Proteinase
Contractor: Wayne State University
Money Allocated: \$71,785 (FY 73) for three years

Objectives: The objective of this contract is to investigate several aspects of biochemistry and immunology of the purified human acrosomal proteinase. The primary interest is to ascertain whether antisera against this enzyme can block fertilization. Secondly the relationship of enzyme activity to the process of semen liquefaction and to the presence of naturally occurring enzyme inhibitors will be investigated.

Major Findings:

(1) The contractor has developed affinity chromatography methods for the purification of acrosin.

(2) The acrosin inhibitors associated with human sperm appear to be derived from seminal plasma as suggested by Zaneveld (1972). However, the distribution of the various acrosin iso-inhibitors in sperm is different from that in seminal plasma. This suggests that certain of the iso-inhibitors in seminal plasma are selectively adsorbed by the spermatozoa.

(3) Individual human ejaculates have been examined for sperm acrosin activity and acrosin inhibitor levels in seminal plasma. All ejaculates analyzed thus far (20 ejaculates) show distinctly measurable amounts of sperm acrosin activity in neutral sperm suspensions. Acidification of these sperm suspensions to pH 2.7 always resulted in a large increase in acrosin activity. The observed amounts of acrosin inhibitor in seminal plasma indicate that this fluid contains a generous excess of acrosin inhibitors.

Significance to Biomedical Research and Program of the Institute: Sperm acrosomal proteinase facilitates ovum penetration by the spermatozoon. The work to be carried out on this project is directly related to the published purpose of the Contraceptive Development Branch to support research dealing with fertilization inhibition.

Proposed Course: This 3-year contract should fulfill the stated objectives.

NICHD ANNUAL REPORT
July 1, 1974 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Control of Sperm Fertilizing Capacity

Contractor : The Worcester Foundation for Experimental Biology
Money Allocated : \$43,425 (FY 73); \$55,000 (FY 74)

Objectives: The investigator plans to 1) establish why seminal plasma vesicles decapacitate spermatozoa, while other vesicles, such as, phosphocholine and RBC ghost vesicles, do not exert this inhibitory effect on sperm potential for fertilization; 2) determine the specific mechanism of action in the sperm cell of the active agent in seminal plasma vesicles responsible for inhibiting sperm fertilizing capacity; and 3) apply what has been learned of this physiological anti-fertility mechanism to the suppression of male fertility.

Major Findings: 1) Fraction I and II vesicles were demonstrated to decapacitate rabbit spermatozoa. Thus, both classes of vesicles in seminal plasma have decapacitation activity. 2) Rabbit spermatozoa decapacitated by Fr I and II became recapacitated when inseminated into the uterus of a doe about 5-6 hours before ovulation. Inhibition of sperm fertilizing capacity by these vesicles is therefore reversible. 3) Fraction I and II vesicles isolated from rabbit seminal plasma inhibited capacitation by rat spermatozoa in vitro. This demonstrates that the fertilizing ability of sperm cells from mammalian species, other than rabbit, can be hindered by these vesicles. It is suggested decapacitation may be of wide occurrence among mammals. 4) Evidence was obtained revealing capacitation is necessary for sperm penetration of the vitelline membrane of an egg. It had been previously suggested by some workers that capacitation was required only for penetration of the zona pellucida. 5) Erythrocyte ghost and phosphocholine vesicles did not show decapacitation activity. 6) Elevation of Ca^{++} levels to 150 μ g/ml in suspensions of uterine capacitated rabbit spermatozoa enhanced the fertilization rate obtained in does following oviductal insemination. But Ca^{++} ions did not prevent decapacitation under the conditions investigated. 7) Disc gel electrophoresis in the presence of sodium dodecyl sulphate revealed that both Fr I and II vesicles have complex polypeptide constitutions (>10 bands). Two prominent bands, corresponding to one small and one large polypeptide, were present in vesicle electropherograms. 8) An active ATPase is present in rabbit seminal plasma in the vesicle fractions. It has an optima near pH 8.0 and $K_m = 0.00053$ M for Fr I. 9) Fraction II vesicles labelled with ^{125}I bound to uterine sperm cell preparations (containing leucocytes), but they did not bind to ejaculated spermatozoa.

Significance to Biomedical Research and Program of the Institute: The study of capacitation and decapacitation is directly related to the published purpose of the Contraceptive Development Branch to support research of possible fertilization inhibitors.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Role of the Epididymis in Sperm Maturation
Contractor: Pennsylvania State University
Money Allocated: \$30,706 (FY 69); \$58,660 (FY 70); \$65,359 (FY 71);
\$89,736 (FY 72); \$156,685 (FY 73 - 2 years)

Objectives: The objective of this research is to evaluate the nature and concentration of androgens in the sperm environment and their physiological effects on testicular and cauda epididymal sperm.

Major Findings: It appears that the testosterone content of bull sperm is considerably greater than that in cauda epididymal plasma and, hence, sperm entering the epididymis may be fully loaded with steroids. Techniques have been perfected to strip endogenous steroids from sperm without altering their glycolytic rate or level of ATP. It is hoped that use of stripped sperm will enable critical evaluation of the role of steroids in modulating sperm metabolism. Additional studies have shown that the method for measuring ATP turnover is valid for bull sperm. The rate of uptake of external $^{32}\text{PO}_4$ does not limit the rate of ATP labeling. Several androgens at low concentrations ($0.16 \mu\text{M}$) were found to alter sperm metabolism, but for ejaculated cells the responses were inconsistent. Levels of adenine nucleotides in cauda epididymal sperm are similar to those in testicular sperm until the cauda epididymal "semen" is diluted. Upon dilution there is a sharp drop in ATP concentrations of sperm, a rise in ADP level and transitory spikes in AMP and cyclic-AMP levels.

Concentrations (ng/ml) of progesterone in rete testis fluid, cauda epididymal plasma, and seminal plasma averaged (n=10 bulls) 0.95, 5.67 and 1.05, while those for estrogens (17β -estradiol and estrone) averaged 0.010, 0.014 and 0.028.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the stated published purpose of the Contraceptive Development Branch to support studies directed towards the development of new male contraceptives.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Hormonal Control of Sperm Maturation

Contractor : Vanderbilt University

Money Allocated : \$27,527 (FY 69); \$37,083 (FY 70); \$50,394 (FY 71);
\$31,356 (FY 72); \$44,735 (FY 73)

Objectives: This contract is concerned with characterization of the androgen receptors of the rabbit epididymis, and with application of the receptors to evaluation of anti-androgens and to the development of potential blocking agents i.e., modified steroids which would combine irreversibly with the receptor site.

Major Findings: The investigator has demonstrated that two androgen-binding proteins are present in the epididymis of adult rabbits. One of these binding proteins is produced in the testis and is confined to the caput epididymidi. This was shown by experiments which prevented testicular fluid from entering the epididymis (efferent duct ligation and hemi-castration) and by experiments which reduced the production of testicular fluid (artificial cryptorchidism). Castration studies indicated the presence of another androgen-binding protein in the epididymis. When rabbits were castrated for various intervals, all three segments had androgen-binding activity at a time when testicular fluid should no longer be present.

A large-scale study has been performed in the rabbit to see if the androgen-binding activity of epididymal cytosol correlates with testis maturation and epididymal differentiation. These results indicate that little or no binding is present in cytosols from the most immature animals, that binding appears in all three segments of the epididymis at an intermediate stage of development, and that binding is demonstrable only in the caput epididymis in the older animals, comparable to the situation in the sexually mature rabbit.

The demonstration of a true epididymal receptor for androgen has been accomplished by DHT binding studies performed on the epididymal cytosol of rabbits following castration for 3 to 4 days. Bilateral castration is necessary to reveal the presence of the epididymal receptor for androgens.

A number of anti-androgens and testosterone analogs have been studied for competitive binding activity in the "epididymal cytosol" preparation from intact animals, in which binding is due to ABP. Of these compounds, 5 α -methyl-B-nortestosterone, 5 α -methyl DHT and 2-Hg Cl-17 α -me $\Delta^{1,6}$ -T were effective inhibitors of 5 α DHT binding to testicular ABP.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new, male methods of contraception.

Proposed Course: Discontinuation.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Hormonal Control of the Epididymis

Contractor : Bureau of Biological Research, Rutgers University
Money Allocated : \$62,539 (FY 72) for two years; \$32,947 (FY 74)

Objectives: The primary objective of this research is to characterize hormonal maintenance of rat epididymal tissue as evaluated by protein, nucleic acid, glycogen and enzyme activity. The general direction of this proposal involves an attempt to evaluate the effects of selected steroids on certain aspects of the biochemical composition of the rat epididymal caput and cauda, a study of the action of two androgen antagonists on epididymal biochemical compounds, and on fertilizing capacity of spermatozoa.

Major Findings: Following gonadectomy, total protein, cell numbers and cell size decline in caput and cauda epididymis, as well as glycogen and glucose-6-phosphate dehydrogenase activity. Aminotransferase activity, on the other hand, increased 100%. Testosterone, dihydrotestosterone, and dehydroepiandrosterone treatments provided incomplete maintenance to about 50% of normal. In general the androgens maintained tissue glycogen concentration and glucose-6-phosphate dehydrogenase activity. Estradiol effects mimicked the castrated rat.

Steroid combinations (androgen + estradiol) gave little evidence of an epididymal response which differed from that of the androgen alone as to protein, nucleic acids, and enzymes.

Epididymal responses were examined in adult male rats castrated for 30 days, a time when sperm and steroid binding protein have disappeared. Testosterone and dihydrotestosterone at 5 mg/kg restored seminal vesicle and prostate weight, but not epididymal. Nevertheless, the epididymal response was only moderately less than that gained by immediate treatment indicating a good response in the absence of the testis androgen binding protein. Little corrective effect was noted relative to the elevated transaminase enzyme activity.

Epididymal response to cyproterone acetate and to Schering #13521 was tested. Compositional changes were modest.

Significance to Biomedical Research and Program of the Institute: The study of epididymal function is one of the stated goals of the contraceptive development program.

Proposed Course: Discontinuation. The stated objectives of the contract will be fulfilled after this contract year.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Androgen Receptors in the Testis
Contractor: The University of Texas Medical Center
Money Allocated: \$96,706 (FY 73 for 2 years)

Objectives: The investigator proposes the following studies:

- 1) Demonstration of the presence of androgen receptors in the testis.
- 2) Localization of the receptors.
- 3) Characterization of the androgen receptors and study of the relationship between soluble and nuclear receptors.
- 4) Study the influence of FSH and testosterone on testicular concentrations of androgen binding protein.

Major Findings: The investigator has established that there is a specific testicular androgen binding protein (ABP), which is secreted from the seminiferous tubular epithelium into the testicular fluid and then passes to the epididymis. The binding protein has the highest affinity for testosterone and dihydrotestosterone. It differs from the more classical steroid "receptor" proteins in that, although of high affinity, it has a very rapid dissociation rate constant. In this respect, it is similar to the progesterone receptor in rat uterus and the estrogen receptor in chick oviduct.

After hypophysectomy, the testicular level of the high affinity component rapidly declines. FSH administration immediately following hypox considerably slowed the decline. Daily testosterone treatment immediately following hypox resulted in maintenance of the androgen binding protein concentration. In cryptorchidism, the level per testis is not elevated compared to normal animals, but the concentration per mg protein is double that from normal testes. This information tends to rule out advanced germinal cells as the source of the binding protein and is consistent with the concept that Sertoli cells are perhaps the source.

Significance to Biomedical Research and Program of the Institute: An understanding of the cellular sites of binding and the nature of the interaction between androgens and specific receptor molecules will allow for a more rational assessment of androgens and anti-androgens as male contraceptive agents and is related to the stated goals of the program.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Morphological and Experimental Studies on Spermatogenesis, Sperm Maturation, and Means of Inducing Reversible Infertility in the Male
Contractor: Harvard Medical School
Money Allocated: \$66,656 (FY 69), \$59,944 (FY 70), \$68,151 (FY 71)
\$71,702 (FY 72), \$60,000 (FY 73), \$60,000 (FY 74)

Objectives: This research is centered upon the use of modern, high quality ultrastructural techniques to investigate key issues of testicular and spermatozoal morphology function. One project deals with topics in spermatogenesis. The investigators are studying the significance of the chromatoid body and aggregates of spermatocytes within the seminiferous tubules, electron microscope radiographic studies of RNA and protein synthesis in spermatocytes and spermatids and, by the use of mitotic blocking agents, interfering with specific developmental events in spermiogenesis in order to learn more about the function of micro-organelles found in germinal cells. The investigators are likewise assessing the influence of androgens on epididymal function.

Major Findings:

1. Ligation of ductuli efferentes is followed by a decrease in height of the epithelium and an even more striking decrease in overall diameter of the initial segment of the ductus epididymis. EM show a persistence of those cellular activities and organelles associated with absorptive function of the epididymis but there is a marked depression of the sparsely granulated endoplasmic reticulum. The results suggest that the initial segment is highly dependent on androgens coming via ABP.

Local application of androgens or antiandrogens was without effect on epididymal function.

2. The contractor has re-examined the blood-testis barrier in experiments involving long-term (4 hrs) infusion of various tracers. Efforts to open the barrier through infusion of hypertonic lithium chloride or urea solutions were not successful. Although some detachment of spermatogonia was observed, the junctional complexes between Sertoli cells remained intact.

3. Utilizing techniques worked out for separation of cells from the gastric mucosa the investigators were able to obtain enriched fractions of spermatocytes and of spermatids prior to elongation.

4. Examination of Sertoli cells from a variety of pathological states suggests that they are not as injury-resistant as has been previously postulated. The accumulation of watery vacuoles seems to be a nonspecific response of Sertoli cells to a variety of insults.

Significance to Biomedical Research and Program of the Institute: Studies of these topics are deemed highly relevant to the purposes of the contraceptive development program.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Metabolism of Sperm Nuclear Proteins During Spermiogenesis
Contractor: Harvard Medical School
Money Allocated: \$42,835 (FY 73)

Objectives: The nuclear protamine, rich in cysteine residues, undergoes oxidation of free sulfhydryl groups to form intermolecular disulfide bridges thus becoming a complex keratinoid network stabilizing the genome. The initial phase of disulfide bond formation is proposed to occur during nuclear condensation of the spermatid and is followed by a more gradual transition during maturation of the spermatozoa in the epididymus.

The objective of this proposal is to verify, by amino acid analysis, that mouse and rabbit sperm nuclear protamine are rich in cysteine residues. With this basic information an intensive biochemical study will be undertaken on the formation of protamine disulfide bonds which is suggested to occur during epididymal maturation of spermatozoa and a function of the epididymal environment.

A further study is planned to test the validity of the hypothesis that initial condensation of the spermatid chromatin is induced by formation of disulfide bonds at the carboxy-terminal and amino-terminal cysteine residues of the protamine molecule. Thus, progressive formation of additional disulfide bonds induced during epididymal maturation of the sperm occurs primarily between internal cysteine residues.

Major Findings: Protamine(s) were isolated from mouse spermatozoa recovered from the caput/corpus epididymis, cauda epididymis, and vas deferens. The basic proteins extracted from epididymal and mature spermatozoa comigrated as single bands when subjected to urea-polyacrylamide gel electrophoresis, thus confirming the presence of protamine in immature spermatozoa. Amino acid analyses of protamine preparations demonstrated that the principal amino acids were arginine (47.2%), histidine (12.2%), serine (12.2%), and Cysteine (9.8%). When examined by polyacrylamide gel electrophoresis, the aminoethylated protamine exhibited two distinct bands, both having electrophoretic mobilities greater than the original non-aminoethylated protein.

The protamine of rabbit sperm was also extracted and purified. Both the non-aminoethylated and aminoethylated protamine gave a single protein band when subjected to urea-polyacrylamide gel electrophoresis. The amino acid analyses of rabbit protamine revealed that the principal amino acids were arginine (54.4%), cysteine (12.7%), and serine (6.2%). In contrast to mouse protamine(s) rabbit protamine was virtually devoid of histidine and appears to be similar to protamines isolated from sperm of the bull, ram, boar, and stallion.

Significance to Biomedical Research and Program of the Institute: A study of protamines and their chemical modification during spermatogenesis and

epididymal maturation of spermatozoa could provide basic information required to elucidate mechanism of action of potential anti-spermatogenic agents and is related to the stated goals of the program.

Proposed Course: Discontinuation. The study will be supported on a Program Project Grant.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural and Electron Cytochemical Studies of Mammalian Spermiogenesis
Contractor: The University of Texas
Money Allocated: \$35,875 (FY 69); \$58,230 (FY 70); \$64,282 (FY 71); \$82,127 (FY 72); \$88,533 (FY 73)

Objectives: The research project involves studies of 1) the formation of the manchette in the spermatids during spermiogenesis, by electron microscopy and cytochemistry at the electron microscopic level (ATPase, succinic dehydrogenase, alkaline and acid phosphatases, thiamine phosphatase, aryl sulfatase and β -glucuronidase; 2) the inhibition of microtubule formation in the manchette (colchicine, cold shock, inhibitors of protein and RNA synthesis; 3) the ultrastructure and cytochemistry of seminal vesicles, bulbourethral gland and prostate.

Major Findings: 1) Chronic, daily injections of Colcemid at sub-toxic doses leads to sterility in male Chinese hamsters within 35 days. The absence of damage to the seminiferous tubules when equivalent doses of lumi-colcemid are administered suggests that loss of germinal cells in the seminiferous tubules after Colcemid injections is due to disruption of microtubules in the Sertoli cytoplasm leading to loss of Sertoli-germ cell contact. 2) Cytochalasin B interferes with a number of cellular processes including microfibrils involved in cell contractibility. Studies with the drug suggest the microfibrils are inhibited leading to multi-nucleated spermatids with multiple acrosomes. This is apparently brought about by disruption of intercellular bridges between spermatids which are maintained by marginal bands of microfibrils around the bridges. 3) Light and electron microscopic studies of the cytochemical characteristics of the Sertoli cells indicate Sertoli cells play a role in maintaining the blood-testis barrier. 4) Studies of the fibrogranular complex, which appears to originate from dense granules in ductuli efferentis of 3-day old Chinese hamsters at the stage when the lumen of the duct begins to appear, indicate that it may be the source of ciliogenesis.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch regarding the development of new male methods of contraception.

Proposed Course: Discontinuation. Majority of the stated objectives have been achieved.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural Changes in the Sertoli Cells in Rats under Various Hormonal Conditions

Contractor: University of Texas Medical School, Houston

Money Allocated: \$126,804 (FY 72) for three years

Objectives: The objective and scope of the present study is to conduct a detailed investigation of the ultrastructure of mature and developing Sertoli cells of rats under various experimental conditions. Specific emphasis will be placed on the possible hormonal interaction which may be operative in Sertoli cells. In vivo and in vitro studies will be made of testes from developing and adult animals deprived of gonadotropic hormones, replaced with gonadotropins or sex hormones.

Major Findings:

- 1) The effects of estrogen treatment and of hypophysectomy on the blood-testis barrier in rats were examined with the electron tracers lanthanum hydroxide and horseradish peroxidase. In those animals treated with estrogen for 12, 20, 30 and 40 days and in those hypophysectomized for 10, 20, or 30 days, no effect of treatment was observed on the specialized Sertoli cell junction, and the blood-testis barrier remained intact.
- 2) Ultrastructural alterations of the peritubular connective tissue associated with the seminiferous tubules of the rat was examined in animals hypophysectomized 20, 30, and 40 days or treated with estrogen benzoate for 20 or 30 days. By the twentieth day following hypophysectomy, profiles of the noncellular components contained within the peritubular connective tissue appear as undulating bands. The basal lamina in the 20-day post-hypophysectomized rats form folds which indent into the cytoplasm of germinal epithelial cells. The end-to-end junctions associated with the thin lateral edges of the myoid cells are maintained in their contiguous positions. In contrast, the 30- and 40-day post-hypophysectomized animals have a more highly disrupted peritubular connective tissue. Estrogen benzoate treatments produced ultrastructural alterations in the peritubular connective tissue similar to the results observed in hypophysectomized animals. In addition, some of the myoid cells in the estrogen-treated rats contained bizarre shaped nuclei not observed in hypophysectomized animals.

Significance to Biomedical Research and Program of the Institute: Understanding of the regulatory function of the Sertoli cells may suggest approaches to interference with seminiferous tubule function. The study is clearly relevant to the goals of the program.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Dynamics of Spermatogenesis: Quantitative Morphological and Physiological Characterization of Isolated Meiotic Cells.

Contractor: The Johns Hopkins University

Money Allocated: \$84,236 (FY 73 for 2 years)

Objectives: This is a proposal to isolate, separate, and characterize meiotic germ cells in rat testes using the lg sedimentation system. Uptake and retention of radioactive steroids (particularly ^3HT) by various types of meiotic cells which have been isolated by gradient analysis will be studied. Further, efforts will be made to maintain isolated meiotic cells in tissue culture and to determine if differentiation of these cells occurs.

Major Findings: The major emphasis during the first six months of this study has been directed at: (1) isolating and identifying the cells liberated from testis, (2) developing the unit gravity sedimentation technique for the separation of the different early germinal cell stages, and (3) validating the identification of certain cell types which are separated by the unit gravity sedimentation.

Classification of germinal cell stage was made on the basis of cytological characteristics following Giemsa staining. Initially dissociation of cells from teased tubules in a trypsinizing flash was accomplished with 5 mM EDTA in calcium and magnesium free phosphate buffered saline. Although this procedure liberated individual cells, it resulted in a considerable amount of inviable cells. A higher number of cells and a higher percentage of viable cells were recovered when the testes were dissociated with trypsin. However, only a small number and percentage of spermatogonia were identified when the cell preparations were stained with Giemsa and the different germinal cells present in the preparations counted. Similar results were observed in testes dissociated from animals ranging in ages from 10-25 days. Preparations of germinal cells liberated with EDTA, in contrast, contained a large percentage of viable spermatogonia. However, if spermatogonial cells were dissociated with EDTA and exposed to Trypsin solution, a high incidence of spermatogonial destruction resulted. This result suggests that the spermatogonial cells are exceptionally sensitive to Trypsin and probably are destroyed subsequent to their being liberated from the tubules. These two methods of dissociation, therefore, have particular advantages for studying the isolating of certain types of germinal cells. In general, however, the Trypsin treatment appears to be more gentle and complete for dissociating and intratubular cells of the testis.

The gradient analyses of the dissociated testes of 20 day old rats clearly demonstrated that cells at different stage of meiosis sediment at different positions in the BSA gradient and are considerably enriched over the concentration of these cells in the starting preparations. Preleptotene sperm-

atocytes and spermatogonia were localized at the top of the gradient and the larger pachytene cells were localized at the bottom. To further validate the relationship between cell type and position in the gradient, testes of various aged rats (10-25 days of age) were dissociated, submitted to unit gravity sedimentation, the different cell types in the fractions were analyzed following Giemsa staining. The results indicated that with increasing age new populations of cells with a larger size are liberated from the testes. In many cases, highly purified meiotic cells (spermatogonia, resting spermatocytes) were obtained from dissociated testes of the young rats without being submitted to unit gravity sedimentation.

Due to the presence of many more cell types in the adult, testes isolation of any class of premeiotic germinal cells from the adult testes was much less efficient than when cells were removed from the immature rat testes.

Significance to Biomedical Research and Program of the Institute: The experimental approach to studying spermatogenesis presented has considerable implication for the development of fertility control methods in the male, which is directly responsive to the stated goals of the program.

Proposed Course: This is expected to be a continuing contractual effort leading to development of new contraceptives. It is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural and Immunological Investigation of Spermatogenesis, Sperm Transport, and Fertilization in the Mammal
Contractor: University of Miami
Money Allocated: \$92,300 (FY 70), \$58,275 (FY 71), \$78,025 (FY 72)
\$59,438 (FY 73)

Objectives: The objective of this research project is to study the effects of antibodies on spermatozoa in the female tract of mammals, using bivalent and univalent fragments of guinea pig and goat anti-rabbit semen antibody.

The investigators will examine the fertilizing capacity and transport of antibody-pretreated spermatozoa following intrauterine artificial insemination, in an attempt to establish whether antibodies can inhibit spermatozoan passage from the uterus through the tubes to the site of fertilization. In addition, intratubal and/or in vitro insemination experiments with univalent and bivalent antibody treatment inhibits capacitation and whether antibody can inhibit the fertilizing capacity of capacitated sperm. Anti-sperm antibody inhibition of sperm head enzymes thought to be responsible for ovum penetration will be tested by preparing extracts of these enzymes and examining their activity on appropriate substrates in the presence of univalent and bivalent antibodies.

Major Findings: Testing for an antifertility effect of immunization with purified rabbit sperm hyaluronidase has been undertaken. Female rabbits were injected with purified hyaluronidase in Freund's adjuvant emulsion and later artificially inseminated. Sera were subsequently examined for rabbit sperm hyaluronidase inhibiting action and precipitating (immunodiffusion) action with rabbit sperm hyaluronidase. Control animals gave 69% fertilization versus 28% fertilization in the immunized group. No hyaluronidase antibody was detected in sera of the control. However, sera of all but one of the immunized rabbits inhibited rabbit sperm hyaluronidase activity. Interestingly, only two of the sera were strongly active and only these produced precipitin bands in immunodiffusion. No obvious correlation between circulating antibody titers and fertility among the individual rabbits was apparent. This suggests that circulating antibodies may not be directly responsible for such infertility as may be present. One alternative explanation would be inhibition through a cellular immune system, e.g., immune lymphocyte-sperm interaction, possibly resulting in release of spermatotoxins.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies which utilize immunological techniques to study reproductive processes.

Proposed Course: Discontinuation.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Androgen Polydimethyl-siloxane Implants: Metabolic Fate of Testosterone and Contraceptive Efficacy of Different Androgens.
Contractor: The Johns Hopkins University
Money Allocated: \$64,256 (FY 73)

Objectives: The investigator proposes to study the effect of testosterone released by PDS implants in different amounts on spermatogenesis, testosterone production rates, plasma testosterone and ICSH concentrations, and accessory sex organ size and morphology in monkeys. The objective is to compare the efficacy of PDS testosterone implants to create azoospermia with elevating plasma testosterone and causing accessory sex organ hypertrophy.

Major Findings: Thirty-six adult male rhesus monkeys were assigned to one of six treatment groups receiving 0, 9.4, 16, 34, 67, or 150 μ testosterone (T)/lb body weight/day via polydimethylsiloxane (PDS) implants. Paired testes weight, sperm/g testis, sperm/paired testes, and sperm/g epididymis showed a biphasic response to increasing testosterone therapy. Sperm/paired testes and sperm/g cauda epididymis was reduced 6 fold in rhesus receiving 34 ug T/lb/day. None of the rhesus was azoospermic. Additionally, both prostate and seminal vesicle weight were increased in animals receiving the predicted contraceptive dose of testosterone (34 ug T/lb/day). Although incomplete, these results suggest that testosterone does not depress pituitary gonadotrophin secretion as effectively in male rhesus as in rabbits. Provided this is true, it suggests that endogenous testosterone secretion is not suppressed as completely as in rabbits. Therefore, plasma testosterone and consequently accessory sex organ size might be elevated due to the additive effect of testosterone emanating from both the PDS implants and from the steroid secreting elements of the testis. Measurement of plasma LH and testosterone concentration, yet to be completed, will verify or refute this hypothesis.

Significance to Biomedical Research and Program of the Institute: Development of new techniques for the control of fertility in male is one of the stated goals of the program.

Proposed Course: Discontinuation. The stated objectives of the contract will be fulfilled after this contract year.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Fine Structure and Function of the Accessory Glands of the Male Reproductive Tract.
Contractor: University of Virginia
Money Allocated: \$12,985 (FY 69), \$12,750 (FY 70), \$18,266 (FY 71), \$25,851 (FY 72), \$83,685 (FY 73 for 3 years)

Objectives: This study of the fine structure and function of the male accessory organs is being carried out principally in rats, with particular attention being paid to (1) effects of vasectomy, both proximal and distal, to the point of ligation of the vas; and (2) changes which take place in the Wolffian ducts of rat fetuses, before, during, and after onset of androgen secretion (at daily intervals from day 14 until birth). Electron microscopy and electron microscope radioautography are being used to follow the fate of radio-labeled precursor substances in a study of the secretory activity of the accessory glands.

Major Findings:

- 1) Radioautography: Studies by light and electron microscope radioautography of protein secretion in the prostate and seminal vesicle of the rat have been concluded. Secretory proteins are synthesized in the rough endoplasmic reticulum transported to the Golgi apparatus and secretory vacuoles, and released to the lumen from the apical ends of the cells. In the seminal vesicle, transport occurs very rapidly, exceeding the rate in the prostate and many other glands.
- 2) Vasectomy in the rabbit: Rabbits have been studied at intervals up to six months after vasectomy. The epididymis and vas deferens proximal to the point of ligation underwent progressive distention. Leakage of sperm from the duct system was not detected in most animals. The epithelium of the cauda epididymidis was altered. Many cytoplasmic processes projected from the apical ends of the cells, and increased numbers of vacuoles, lysosomes, and profiles of the smooth endoplasmic reticulum were observed. These changes suggest increased absorptive activity by the epithelium of the cauda epididymidis.
- 3) Effects of cyproterone acetate: Experiments involving the treatment of male rats with 10 mg per day of cyproterone acetate are in progress. After two weeks of treatment, the weight of the epididymis, prostate, and seminal vesicle was greatly reduced, but, interestingly, the histology and cytology of the organs were not yet altered significantly.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Study of Mammalian Spermatozoa
Contractor: University of Houston
Money Allocated: \$14,451 (FY 69), \$15,347 (FY 70), \$15,347 (FY 71),
\$20,047 (FY 73)

Objectives: The objective of the project is to study the subacrosomal region of the mammalian spermatozoon with consideration of that region as a possible target for the development of new contraceptive agents or techniques. The investigator will attempt to develop a reliable technique for selective extraction of subacrosomal material from spermatozoa and to characterize the material biologically, immunologically and biochemically.

Major Findings: Several fractionation procedures have been explored to recover clean sperm heads free of acrosomes and tails and the detachment and recovery of the hair-like "perforatorium" from clean heads of hamster sperm. Trypsin treatment of hamster sperm as a means of cleaving heads from tails has provided dramatic results. This treatment not only disrupts the acrosomes, but also fractures whole tails from sperm, thereby simplifying subsequent separation. Clean head fractions are obtained in a cesium chloride gradient at low centrifugal speeds.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to support research on potential areas of male contraception.

Proposed Course: Discontinuation. The study is not providing unequivocal data.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Immunologic Localization of Prostaglandins in the
Reproductive Tract of Rabbit
Contractor: Yale University School of Medicine
Money Allocated: \$39,850 (FY 72) \$14,186 (FY 73).

Objectives: The objective of this research is to study the localization of prostaglandins in the uterus, oviduct and on sperm during the capacitation process. Antibodies to prostaglandins will be coupled with peroxidase incubated with the respective tissues and subsequently reacted with diaminobenzidine. The last reaction produces electron dense material which can be subsequently studied with EM. The localization studies may provide an answer for the site of action or synthesis of prostaglandins at the cellular level.

Major Findings: Methods were developed for identifying the reaction products between prostaglandins and their antibodies. The methodology was applicable for the demonstration of PG's on sperm heads and flagella, but could not be applied to such PG-rich tissue as seminal vesicles.

Significance to Biomedical Research and Program of the Institute: The projected study on the localization of prostaglandins is directly related to the stated interests of the Contraceptive Development Branch in this group of substances.

Proposed Course: Discontinuance.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Formation of Dihydrotestosterone in Male Sex Organs
and Transport of Steroid Androgens from the Testis to
the Epididymis

Contractor: University of Trondheim

Money Allocated: \$95,860 (FY 74) for two years

Objectives: The contractor will conduct studies dealing with the formation and transformation of testicular androgens and their transport to the epididymis. He will ascertain the presence of specific binders for the androgens in different testicular compartments. He will likewise study the testicular enzyme system responsible for converting testosterone to dihydrotestosterone.

Significance to Biomedical Research and Program of the Institute: This project is directly relevant to the contraceptive program since epididymal physiology has been identified as a particularly important topic for the exploration of contraceptive approaches.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptive technology.

NICHD ANNUAL REPORT
July 1, 1973 through July 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Assay of Progesterone Receptor Activity in the Guinea Pig Uterus
Contractor: Milton S. Hershey Medical Center
Money Allocated: \$20,694 (FY 71), \$23,544 (FY 72), \$83,519 (FY 73, for two years) \$11,130 (FY 74)

Objectives: The biological action of progesterone on the uterus may be initiated by the interaction of this steroid with a specific intracellular receptor protein. In the uterus of the castrate estrogen treated guinea pig specific cytoplasmic receptors for progestins have been identified. The purpose of this study will be to characterize these receptors in order to determine the best technique for measuring their binding capacities. A rapid assay technique will be developed for uterine cytosol protein binding activity. This assay will then be used to search for agents which both block progesterone binding to the cytosol receptor and which have relatively little progestational activity. From experience with other steroid receptor systems, it is anticipated that these agents will be antiprogestational and may therefore be useful as contraceptive agents.

Major Findings: The nuclear binding component can be physically distinguished from that in the cytosol. Studies indicate that both dialysis and 0.4M KCl markedly increase the half-life of the cytosol receptor. Dialysis does not influence the half-life of the nuclear binding component. High ionic strength buffers decrease the sedimentation of both the cytoplasmic and nuclear receptors.

Nuclear receptor binding of a number of biologically active progestins was very similar to cytoplasmic binding. The 17-acetoxy progestins bound poorly to both receptors. Thus, the difference in the relative binding and biological activities cannot be explained by a difference in the nuclear receptor protein.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies on the binding characteristics of steroid receptor systems.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Studies on Steroid Hormone Receptors and their Role in Reproductive Biology

Contractor: Mayo Clinic

Money Allocated: \$111,121 (FY 73) for 2 years

Objectives: The objectives of this research project are as follows: (1) purify and study the binding properties of the estrogen and progesterone receptors from cow uteri, (2) investigate the interaction between receptor proteins and the DNA and chromatin; (3) investigate the interaction among estrogens and progestins and their competitive binding to their respective receptors.

Major Findings: (1) Efforts are under way to purify the progesterone receptor from cow uteri. As with other steroid receptors, the instability of the binding protein is a problem. Glycerol and CaCl_2 have a stabilizing effect on the receptor protein. The binding of progesterone in cytosol preparations from cow uteri was found to be comparable to previous observations on hamster and human uteri. The majority of bound progesterone sediments on sucrose gradients as a 7S complex. The binding component is highly specific for progesterone as determined by competitive binding analysis. (2) A number of synthetic and naturally-occurring steroids were studied for competitive binding to two progesterone receptors, one in chick oviduct and another in human uterus. The results show a good correlation of relative (to progesterone) binding affinity with progestational activity as measured by subcutaneous Clauberg assay. 17α -Ethinyltestosterone and closely related compounds compete very well in binding to both receptors; 19-nor derivatives show dramatic enhancement of binding affinity to the human receptor. Of the substituted progesterones tested, 21-fluoro- and 6 α -fluoro- progesterone competed best with progesterone for binding to both receptors. 17α -Acetoxypregesterone and the substituted 17α -acetoxypregesterones tested were fairly good competitors for the human receptor but did not compete at all for the chick.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the steroid binding substances in reproductive tract tissues.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Study of Progesterone-Binding Proteins as a Tool for the Detection of Progesterone Antagonists and Agonists
Contractor: Institute of Medical Research of the Toledo Hospital
Money Allocated: \$74,675 (FY 72), \$89,353 (FY 73), \$93,480 (FY 74)

Objectives: The purpose of this project is to prepare, isolate, purify and characterize a standard cell-free fraction containing receptor proteins from the uteri of rats, rabbits and guinea pigs incubated with ³H-progesterone and from fresh untreated uteri in order to develop a rapid screening system for the detection of progesterone antagonists and agonists.

Major Findings: The contractor has developed systems of buffers and electrolytes which optimize the extraction and stabilization of the 7S progesterone receptor complex from uterine homogenates in a form suitable for column chromatography. Results suggest that there are at least 3 major configurations which the receptor can take. These have sedimentation coefficients of 4.5-5.0S, 5.5-6.0S, 6.5-7S. The sedimentation coefficient derived in an analytical sucrose density gradient analysis is dependent upon the presence of electrolytes and the concentrations of both the receptor and the ligand. Limited data indicate that the cytoplasmic estrogen receptor system behaves similarly.

For molecular sieving, Dextran appears to be the gel of choice. Dilution of the receptor solution upon chromatography presents problems by reducing the yield, but methods were developed for concentrating solutions utilizing hollow fiber technology.

These studies have pointed the way to the development of a procedure for the large scale preparation of 7S receptor (hopefully ligand-free) from the guinea pig uterus. The guinea pig appears to be the species of choice due to receptor stability and minimal glucocorticoid binding.

Studies on the 7S ↔ 4S conversion with high ionic strength solutions and upon chromatography indicate that there may be an aggregation process in the formation of the 7S complex. However, there are no data which can rule out conformational changes. Reformation of the 7S complex from high ionic strength solution may be used as an assay for nonsteroid binding components of the 7S receptor if these exist.

During pregnancy, the configuration of the receptor seems to change, for very little 7S binding is detectable. Limited data indicate that the 4.5S pregnancy binder is similar to proteins found in plasma. It appears that part or all of the 7S receptor complex may be missing during pregnancy.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of progesterone binding substances in the uterus.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Estrogen-Receptor Substances of Oviduct Tissue
Contractor: University of Chicago
Money Allocated: \$45,413 (FY 69), \$29,815 (FY 70), \$31,944 (FY 71),
\$36,265 (FY 72), \$108,423 (FY 73 for two years)

Objectives: The investigators are studying the molecular mechanism of estrogen activity in oviduct tissue by biochemical and autoradiographic determination of the presence, intracellular localization, and chemical nature of specific "estrogen receptor" proteins.

Major Findings: Quantities of the purified estrogen receptor have been prepared. Efforts are underway to produce antisera against the receptor and to investigate their biological action.

Significance to Biomedical Research and program of the Institute: This particular study is an important step in understanding hormone and possibly anti-hormone action at the cellular level and is directly relevant to the purposes of the Contraceptive Development Program.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHE ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Model for Studying Inhibition of LH Action on the Corpus Luteum
Contractor: Mayo Clinic and Mayo Foundation
Money Allocated: \$36,924 (FY 71), \$68,620 (FY 72), \$59,740 (FY 74) for two years

Objectives: A model system has been developed for studying the interaction of human luteinizing hormone (LH) with cells of the corpus luteum using slices of pseudopregnant rat ovaries. The object of this contract is to further validate this model, to define the structural requirements of the LH molecule, to define the requirements of the luteal cell for this interaction, and to seek inhibitors of this interaction. If such inhibitors are found, they may be highly specific abortifacients or contraceptives.

Major Findings:

- (1) The LH-HCG receptor has been isolated from rat corpora luteal tissue. The purified receptor retains binding activity and specificity, but has a lower dissociation constant than the particulate receptor. The purified receptor appears homogeneous on disc gel electrophoresis and gives a single precipitin arc on immunodiffusion using three antisera raised in goats and rabbits. Antisera have been demonstrated to block LH binding to receptor both in vivo and in vitro.
- (2) Use of the antisera to the receptors in immunodiffusion tests with Triton-X-100 extracts of rat liver, spleen and kidney indicated the presence in these nontarget tissues of material that gave an immunologic reaction of identity.
- (3) Antibodies to HCG and HCG β are noncompetitive inhibitors of HCG binding to the receptor. Anti-HCG α inhibits binding but the kinetics are neither competitive nor noncompetitive

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research in the relationship between LH and the function of the corpus luteum.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Biochemistry and Mechanism of Action of Human
Gonadotropins
Contractor: Cornell University Medical College
Money Allocated: \$58,736, (FY 72), \$45,377 (FY 74)

Objectives: The objectives of this project are: (1) to isolate and characterize the gonadotropin binding material from the subcellular fractions of the gonads and (2) to study the interaction of gonadotropins (LH and FSH) with the binding material.

Major Findings: The gonadotropin receptor from bovine corpora lutea can be utilized as a reliable and sensitive radioreceptor assay for hCG. Through the utilization of this assay, it has been demonstrated that in the pre-implantation rabbit blastocyst fluid there is a substance with binding characteristics similar to hCG or LH. The nature of this substance is being explored.

Significance to Biomedical Research and Programs of the Institute: Study of gonadotropin receptors may lead in the future to the understanding of the mechanism of action of gonadotropins and formulation of new contraceptive technology. As such it is directly related to the published purpose of CDB.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Steroid Hormone Binding in Reproductive Organs
Contractor: Yale University
Money Allocated: \$39,141 (FY 70), \$58,500 (FY 71), \$67,692 (FY 72)
\$34,549 (FY 73)

Objectives: The objective of this research project is to study various aspects of the interaction of contraceptive drugs and potential contraceptive agents with steroid binding molecules in organs concerned with reproduction. The investigator will continue studies directed towards in vitro comparisons of estrogen binding molecules from the rat uterus, vagina, anterior pituitary, and hypothalamus. The affinity of estradiol for binding to macromolecules from the supernatant fraction of these organs will be determined after in vitro incubation, and various drugs will be examined for their ability to compete with tritiated estradiol for macromolecular binding. To facilitate these studies, the investigator will attempt to develop a gentle and rapid purification procedure for estrogen binding macromolecules utilizing as the separating medium, estradiol reversibly linked to a standard chromatographic resin.

Major Findings: Recent studies indicate that the liver has high affinity estrogen binding macromolecules. The liver estrogen binders are absent in immature animals. Presence of these binders may help to explain the known effects of estrogens on the liver.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the steroid binding mechanism in reproductive and central nervous system tissues.

Proposed Course: Discontinued. Study of liver estrogen binders is the responsibility of another Branch.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Impeding Estrogens as Contraceptives - Mechanism of Action
Contractor: Boston University School of Medicine
Money Allocated: \$56,572 (FY 73)

Objectives: The hypothesis that contraceptive agents can be developed, which by competing for cytoplasmic estradiol receptor, interfere with endometrial development, has been, at least in part, proven. It is proposed that more conclusive evidence for the mechanism of contraceptive action of impeding estrogens be obtained by investigating a series of trihydroxylated epimeric estrenes. Preliminary evidence supports the postulate that anti-conceptive activity and estrogenicity are not necessarily parallel effects, and that a more significant relationship exists between anti-conception and the ability to interfere with estradiol binding in the uterine cells.

Major Findings: Cytosol association and dissociation constants have been determined for estradiol, estriol, 16-epiestriol, 11 β -hydroxyestradiol, and 16 α -estradiol. Particularly interesting appears to be 16 α -estradiol, which has an equilibrium constant (K_A) one-half that of estradiol. The association rate constant is at least equal to and appears to exceed that of estradiol, while the dissociation rate constant exceeds by a factor of 3 even the dissociation of the impeding estrogens. This is a particularly interesting situation, in that here is a substance which is lacking the 17-hydroxyl function, but is otherwise analogous to estriol through its 16 α -hydroxyl. It displays a high affinity for the receptor, but with a very rapid dissociation from it.

In vivo, there appears to be such rapid conversion of the 8S receptor complex to the nuclear 5S receptor, that dissociation from the 8S receptor as observed in vitro may be of lesser significance. However, no definitive statement regarding any of these relationships can be made until the nuclear 5S dissociation rate constants for all the compounds have been determined.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the stated goals of the Contraceptive Development Branch to support studies directed towards the development of new contraceptives.

Proposed Course: Discontinuation. The objectives will have been realized by the end of the contract period.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Hormonal Requirement for Implantation in the Rhesus Monkey
(Macaca mulatta)

Contractor : University of Wisconsin
Money Allocated: \$78,335 (FY73/2 years)

Objectives: It is proposed to use the egg transfer technique to determine the requirement of ovarian hormones for survival of the blastocysts and implantation in the rhesus monkey. Blastocysts will be obtained from animals that have ovulated and mated naturally or have been treated with gonadotropins. The recipients will be ovariectomized two weeks before use and given daily injections of progesterone prior to egg transfer. If implantation does not occur, recipients will be given estrogen in addition to progesterone.

Major Findings: Six pregnant rhesus monkeys are currently being studied. Three animals were ovariectomized on day 5 of pregnancy and are receiving daily injections of progesterone (5 mg/kg BW) throughout the remainder of gestation. Three additional monkeys are being retained as intact controls. Blood samples obtained from these animals at either daily or twice weekly intervals will be analyzed for monkey chorionic gonadotropin, progesterone and estrogen. It is envisaged that the data from these experiments will provide a basis for future experiments in which blastocysts will be transplanted to ovariectomized monkeys treated with estrogen and/or progesterone.

The utility of the gonadotropin treated hypophysectomized monkey as a predictable source of ova is also being investigated.

Two hormonal regimens have been explored thus far. One of these involved the treatment of hypophysectomized female monkeys for nine days with Pergonal (75 I.U. FSH and 75 I.U. LH) i.m. followed on day 10 by an I.V. injection of the same dosage of hormone. The monkeys were laparotomized on day 10 and again 48 hours following the last gonadotropin injection. The ovaries were examined for the presence of follicles and corpora lutea.

The second hormonal regimen was the same with the single exception that on day 10, 2,000 I.U of HCG were administered rather than Pergonal.

The first hormonal schedule induced a moderate increase in both the number and size of ovarian follicles but no CL were observed. Greater success was obtained utilizing the second sequence of hormones. A total of 14 follicles with a size > 4 mm were observed following nine days of Pergonal treatment. Following HCG, seven fresh corpora lutea were developed.

The above data indicate that the hypophysectomized monkey could be used as a source of ova.

Significance to Biomedical Research and Program of the Institute: Knowledge concerning the hormonal requirements for implantation in the monkey is related to the stated goals of the program.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis and Testing of Antifertility Compounds
Contractor: Stanford Research Institute
Money Allocated: \$108,908 (FY 71), \$82,798 (FY 72), \$83,647 (FY 73)
\$70,000 (FY 74)

Objectives: The purpose of this project is to synthesize a series of side-chain silicon-containing steroidal compounds and test their antifertility effect in animals.

Major Findings: The introduction of silicon into certain steroids markedly affects their biological response and causes a favorable separation of antifertility activity from estrogenic activity. Two compounds, 17 α -dimethyl-t-butylsilylethynyl estradiol and 17 α -triethylsilylethynyl estradiol have shown a seven-fold increase in antifertility potency (postcoitally) in rats, orally, when compared with ethynyl estradiol, but only show 20% and 40%, respectively, of the estrogenic activity in rats. Preliminary metabolic studies indicate significant differences between these silicon containing steroids and ethynyl estradiol with regard to their excretory and tissue distribution patterns. Further biochemical studies may elucidate the mechanism of action of this new class of antifertility agents. Preliminary results indicate that one of these compounds is less estrogenic in the rhesus monkey than is ethynyl estradiol. Both compounds are currently being evaluated in the rhesus monkey as an antifertility agent. Other chemical modifications of the side chain are being pursued in attempts to effect even greater separations of antifertility activity and estrogenic activity. Structure activity studies have been conducted with this series of compounds, and there are good correlations between the observed biological activities and the calculated van der Waals' volumes of the three variable silicon substituents.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Prostaglandin Analogs for Biological Studies
Contractor: University of Chicago
Money Allocated: \$46,675 (FY 71), \$20,943 (FY 72), \$48,502 (FY 73)
\$137,968 (FY 74), 2 years

Objectives: A major objective of this project is to synthesize several of the more promising prostaglandin analogs in small gram-quantities for evaluation as luteolytic agents in monkeys and further biological studies of the effects of these substances on reproductive processes. Another objective of this project is to continue to prepare prostaglandin-like compounds in chemically pure form and in sufficient quantities for evaluation as antifertility agents in animals.

Major Findings: Approximately three grams of 13-dehydro PGF_{2α} is being prepared; 810 mg of pure material is now available and additional quantities are in various stages of processing. Only small amounts (75 mg.) of ent-13-dehydro-15-epi-PGF_{2α} are available at this time. The synthesis of 13-dehydro-erythro-16-fluoro-PGF_{2α} is almost complete. The fluoro atom in the 16-position should increase the pK of the important 15-hydroxyl group and thus exert a profound effect on biological activity.

13-Dehydro-PGF_{2α}, via subcutaneous administration, has been found to be eight times as potent as PGF_{2α} as an antifertility agent in the hamster. Using a bovine corpus luteum receptor preparation which preferentially binds PGF_{2α} (ca. 100 times better than PGE₂ or PGF_{1α}) the demonstrated receptor binding is as follows: 13-dehydro-PGF_{2α} : .15 X PGF_{2α}, ent-13-dehydro-15-epi-PGF_{2α} : .005 X PGF_{2α}; ent-13-dehydro-PGF_{2α} : 2 X 10⁻⁵ X PGF_{2α}. The luteolytic properties of 13-dehydro-PGF_{2α} and ent-13-dehydro-15-epi-PGF_{2α} were further studied in the sheep with ovaries autotransplanted to the neck. Infusion of either compound into the ovarian artery at levels as low as 2.5μg/hr. show that both compounds were highly luteolytic at infusion rates of 10μg/hr. or more for six hours. Whereas both compounds show weak luteolytic activity systemically (infusion rates of 50μg/hr. for six hours) PGF_{2α}, the parent compound, at the same dose, systemically, had no luteolytic effect. This bears out, in vivo, the known resistance of both compounds, in vitro, to the 15-dehydrogenase enzyme.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandin analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Production of New Type of Contraceptives Based on Derivatives of Luteinizing Hormone-Releasing Hormone (LH-RH)
Contractor: Tulane University
Money Allocated: \$51,716 (FY 72) \$71,000 (FY 73) \$90,000 (FY 74)

Objectives: The purpose of this project is to prepare and evaluate biologically decapeptide analogs of LH-RH which involve modifications of various amino acid residues in order to produce an inhibitor of LH-RH (LRF).

Major Findings: Twenty-one LRF analogs have been synthesized. It has been confirmed that the D-Ala⁶ and des-Gly¹⁰ ethylamide modifications alone and combined enhance the activity of LRF and enhance the inhibitory potency of [des-His²]-LRF. It has also been shown that the increased potency (agonists) is due to prolonged action. [Des-His², des-Gly¹⁰]-LRF, ethylamide is active in vivo as an inhibitor of LH release (but not of FSH release) and is able to inhibit ovulation in rats (injection twice a day for six days). The fact that [Leu¹, des-Gly¹⁰]-LRF, ethylamide is also an in vivo inhibitor of LH release is an interesting finding. Further in vivo assays on these and related inhibitors are continuing.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of LRF analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Contract Program for Obtaining, Synthesizing, and Testing Antifertility Compounds
Contractor: Hazleton Laboratories
Money Allocated: \$44,800 (FY 72) \$49,916 (FY 74)

Objectives: The purpose of this project is to study and confirm the anti-fertility activity of an antibiotic mixture in dogs and several species of small laboratory animals and to identify the active agents(s).

Major Findings: It has been determined that an ingredient was present in randomly purchased lots of a commercially available antibiotic premix which was a potent contraceptive agent. Subsequent studies identified the active ingredient as a mixture of quaternary ammonium compounds. Five other quaternary ammonium mixtures of varying composition have canine contraceptive properties. The compounds are mixtures of monalkyl trimethylammonium chloride salts. When the compounds were fed to 25 dogs none became pregnant; control dogs became pregnant. Additional studies demonstrated that when the compounds were given to rats and rabbits a contraceptive effect was only observed with higher doses and then profound gastrointestinal toxicity was observed. A single quaternary ammonium salt (99% purity) was also found to be completely effective in dogs. At present these studies are being extended to determine the period of maximum contraceptive efficacy in dogs. A contraceptive non-human primate study is also being initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel chemical agents for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Blastocidal and Spermicidal Action of Extracts and
Compounds from Endod (Phytolacca Dodecandra).
Contractor: Stanford Research Institute
Money Allocated: \$45,588 (FY 74)

Objectives: The purpose of this project is to isolate, purify and characterize the various blastocidal and spermicidal active components extracted from Phytolacca Dodecandra, including oleanoglycotoxin-A,-B, and -C. These purified components will be evaluated for blastocidal and spermicidal activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel chemical agents for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Development of Inhibitors to Estrogen Biosynthesis
Contractor: Worcester Foundation for Experimental Biology, Inc.
Money Allocated: \$43,634 (FY 70), \$43,634 (FY 71), \$74,745 (FY 72)
\$48,775 (FY 74)

Objectives: The investigator seeks to develop inhibitors of estrogen biosynthesis, in particular specific inhibitors to the aromatase enzyme system which converts androstenedione to estrogens. One of the primary objectives is to obtain evidence that the effective compounds indeed act by virtue of their inhibition of estrogen synthetase activity. Another primary goal is to exploit accumulated structure-activity correlations in the preparation of additional inhibitors with improved performance in the in vitro and in vivo test systems.

Major Findings: The steroid estrogen synthetase inhibitors required for in vivo evaluation have been prepared or will be in hand when required. The prime purpose for preparing these compounds is to determine to what extent they alter estrogen and testosterone plasma levels in the rat and the sheep. Chromatographic procedures have been worked out for separating the inhibitor from these two hormones prior to radioimmunoassay and for assaying testosterone in peripheral plasma of untreated female rats. The levels have not been reported previously and are in the 200-250 pg/ml range.

The best method of injecting the inhibitor to obtain the optimal plasma concentration of inhibitor prior to sampling the rat venous blood is being determined. Intramuscular and subcutaneous injections provide more uniform blood levels with time although higher plasma levels can be achieved with the former method. One or both of these methods will be used for the assays depending on the blood levels required. This in turn will depend in part on how much inhibitor concentration can be tolerated by the radioimmunoassay procedure without necessitating extensive revision of the purification procedures.

The estrogen synthetase system from rat ovary, obtained by long term stimulation with pregnant mares' serum, has now been obtained in a reproducible fashion. In addition the testing procedure for inhibitors using the rat ovary system has been modified so as to now give more reproducible results.

The program for in vivo testing of inhibitors as antifertility agents was continued. 4-Hydroxytestosterone 17-formate, 100% effective when treatment was begun before mating was also 100% effective when given after mating. The corresponding 17-formate was 50% effective post-mating as compared to 100% when treatment was begun before mating under similar conditions (all 12mg/kg/day). 4-Hydroxyandrost-4-ene-3,17-dione was 100% effective with pre-mating administration as compared to 15% effectiveness when administration was begun the morning after mating (50 mg/kg/day).

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the biosynthetic pathways for ovarian steroids with special attention being paid to possible points of inhibition for the purpose of fertility control.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesize and Furnish Steroidal Compounds with Anti-fertility Activity
Contractor: Laval University
Money Allocated: \$110,772 (FY 72) - for two years, \$60,699 (FY 74)

Objectives: The purpose of this project is to synthesize 11-oxa, and 11-aza analogs of certain contraceptive drugs for biological evaluation of their effects on the reproductive system. Preliminary biological evaluation indicated that 11-oxaprogesterone has enhanced ovulation inhibiting activity and reduced progestational activity on subcutaneous administration when compared with progesterone.

Major Findings: Four compounds, 11-oxaprogesterone, 17 α -acetoxy-11-oxaprogesterone, 17 α -ethynyl-11-oxatestosterone and 17 α -acetoxymethylprogesterone have been synthesized in small quantities (50 mg) and submitted for testing. Only one compound, 11-oxa-progesterone, has been tested thus far. Preliminary results in the rat indicate that 11-oxaprogesterone is considerably less active (contrary to the original antioviulatory data in the rabbit) than progesterone, s.c., as an antioviulatory agent. Substantial quantities of two key intermediates, methyl 3 β , 17 α , 20 α -thihydroxy-9-oxo-9,12,seco-11-nor-5 α -pregnan-12-oate, and methyl 3,9,7-trioxo-9, 12-seco-11-nor-5 α -androstan-12-oate, necessary for the preparation of the remaining products, have also been synthesized. The groundwork has also been laid for the completion of the project objectives (the synthesis of 11-oxa-steroids in 100 mg-1.0 gram quantities).

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesis and Testing of Silicon Substituted Steroids as Potential Antifertility Compounds.
Contractor: Research Triangle Institute
Money Allocated: \$30,312 (FY 72), \$49,998 (FY 73)

Objectives: The purpose of this project is to synthesize silicon ring-substituted steroids related to estradiol and to test these steroids for antifertility and estrogenic activity.

Major Findings: The method of introduction of silicon at the 6-position in the steroid nucleus was based on a modification of the Torgov-Smith procedure for the total synthesis of steroids. Three 6,6-dimethyl-6-sila analogs of estradiol 3-methyl ether were prepared (the $\Delta^{8(14)}$, Δ^8 and $\Delta^{8,14}$ unsaturated compounds) and displayed no uterotrophic activity, s.c., at doses of 100 μ g. The latter compound ($\Delta^{8,14}$) showed no significant antiuterotrophic activity at 1000 μ g. Surprisingly, the 6,6-dimethyl-6-sila analog of mestranol was synthesized and displayed no uterotrophic activity at 1000 μ g, p.o., and no postcoital antifertility activity at 5 mg/kg! Attempted O-demethylation of the 6-sila analog of estrone 3-methyl ether with boron tribromide resulted in silicon-aryl cleavage, gave the secosteroid (5,6-Seco-3-methoxy-6,6-dimethyl-6-hydroxy-6-silaestra-1,3,5(10)-trien-17-one). Two additional secosteroids (5,6-Seco-3-methoxy-6,6-dimethyl-6-fluoro-6-silaestra-1,3,5(10)-trien-17-one, and 5,6-Seco-3-methoxy-6,6-dimethyl-6-fluoro-6-sila 17 α -ethynylestra-1,3,5(10)-trien-17 β -ol) were also prepared and are currently being evaluated biologically. The synthesis of 6,6-dialkyl-6-sila progesterone analogs are in progress and should be completed by the end of the contract year.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal compounds for contraceptive utility.

Proposed Course: Termination in the absence of significant biological activity for these compounds.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesis of Potential New Agents for the Control
of Fertility
Contractor: University of Rhode Island
Money Allocated: \$29,660 (FY 72), \$40,879 (FY 74)

Objectives: The purpose of this project is to prepare 9(10→19)abeo-steroids (substances related to 19-nor steroids but having an extra methylene group between ring A and C) and to test their antifertility and hormonal effects in rats.

Major Findings: Although the original synthetic scheme plus several alternate schemes starting from 3-methoxy-9,10-seco-1,3,5(10)-androstatriene-9,17-dione and/or from 1-tert-butoxy-5,6,7,7a-hexahydro-7a-methyl-4-cyanomethylindan-5(4H)-one failed completely to produce the desired 9(10→19)abeo-steroids, the difficulties were fortunately overcome by the advent of the most versatile key intermediate, (±)-1β-tert-butoxy-3α,6,7,7a-tetrahydro-7aβ-methyl-4-methyleneindan-5(4H)-one. The copper catalyzed 1,4-conjugate addition of m-methoxybenzylmagnesium chloride to the above α-methylene ketone was achieved successfully and resulted in the formation of 3-methoxy-17β-tert-butoxy-9,10-seco-estra-1,3,5(10)-trien-9-one. Treatment of the 9,10-seco-steroid ketone with the Wittig reagent, ((methoxymethyl)-triphenyl-phosphonium chloride) followed by subsequent cyclization of the enol ether gave the desired 9(10→19)abeo-steroid. Larger quantities of this key intermediate will now be prepared followed by appropriate transformations to the desired 9(10→19)abeo modifications of ethynyl estradiol, testosterone, norethindrone, and progesterone.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT

July 1, 1973 through June 30, 1974

Contraceptive Development Branch

Contract and Collaborative Research

Contract Title: Synthesize and Furnish Peptide Inhibitors of Luteinizing Hormone-Releasing Factor/Follicle Stimulating Hormone-Releasing Factor

Contractor: University of Wisconsin

Money Allocated: \$35,560 (FY 72), \$17,105 (FY 73)

Objectives: The purpose of this project is to synthesize competitive inhibitors of LH-RH/FSH-RH (LRF) by replacing certain hydrophobic amino acid residues in LRF by unusual amino acids to increase the hydrophobic properties of the amino acid normally present in that position while providing a minimal steric hindrance between the releasing hormone at that position and its receptor. Such competitive inhibitors are potential antifertility agents.

Major Findings: Three resolved dimethyl-L-tryptophane derivatives have been prepared and submitted (300-500 mg) to the Contraceptive Development Branch. These resolved amino acids will be substituted for tryptophane in LRF.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of LRF analogs for contraceptive utility.

Proposed Course: Termination.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesize Novel Derivatives of Progesterone and Testosterone
Contractor: University of Pittsburgh
Money Allocated: \$66,779 (FY 72) \$26,851 (FY 73)

Objectives: The purpose of this project is to prepare unique chemical modifications of certain contraceptive steroids which, due to the introduction of unsaturation and the 10α (retrograde) configuration, will produce changes in the geometry of the steroidal conformation and thus might lead to selective changes in steroidal receptor attachments and therefore to interesting physiological consequences. These steroids will be tested for antifertility activity and other hormonal effects.

Major Findings: dl-17 β -Hydroxy androsta-4,8,14-triene-3-one and its 10α -methyl analog had neither androgenic nor antifertility (female) activity in rats, s.c. dl-14 β -Androsta-4,8-diene-3,17-dione and its 10α -methyl analog displayed neither androgenic, antiandrogenic nor antifertility (female) activity in rats, s.c. 5 α -Androsta-8(14)-ene-3,17-dione and the corresponding 4,8(14)-diene had neither androgenic nor antiandrogenic activity in rats, s.c. 5 α -Pregn-8(14)-ene-3,20-dione showed neither progestational nor antiprogestational activity in rabbits, s.c. Biological investigation of 19-nor androsta-4,8(14), 9(10)-triene-3,17-dione is in progress.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: Termination. All of the contract objectives have been achieved.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Prostaglandin Analogs
Contractor: Research Triangle Institute
Money Allocated: \$63,200 (FY 72) \$60,940 (FY 73)

Objectives: The purpose of this project is to synthesize 10-oxa-prostaglandin analogs lacking the 11-oxygen function (lactones) as well as the corresponding lactols and open chain analogs and to test these compounds for antifertility activity.

Major Findings: The synthesis of the lactones, the methyl esters of dl-10-oxa-11-deoxy-15(S)-15-methyl-PGE₁, dl-10-oxa-11-deoxy-15(R) and 15(S)-PGE₁ have been completed. These compounds did not exhibit antifertility activity (postcoital) in the hamster at the doses tested. The preparation of the lactols from the lactones has not yet been accomplished. The open chain analogs of PGF_{1α}, methyl-10-nor-9,11-seco-9,11, 15(R) and (S)-trihydroxy-13-prostenoate have also been synthesized. Biological evaluation of these compounds are in progress.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandin-like compounds and prostaglandin antagonists for contraceptive utility.

Proposed Course: Termination in the absence of significant biological activity in this series of compounds.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch

Contract: Structure-Activity Studies of Small Peptides which
Release LH and FSH
Contractor: Tulane University
Money Allocated: \$59,171 (FY 72), \$110,570 (FY 73) \$97,414 (FY 74)

Objectives: The purpose of this project is to biologically evaluate new synthetic polypeptides, which may affect the reproductive system, based upon structural modifications of the decapeptide, LH-RH/FSH-RH (LRF)

Major Findings: Analogs of the luteinizing hormone-releasing hormone have been prepared mainly by solid phase procedures, with suitably protected amino acid derivatives.

Cleavage of the completed peptide resin with NH_3 in MeOH followed by deprotection with anhydrous HF gave the peptide amides, whereas cleavage with HF gave the peptide acids.

The peptides were purified over Bio Gel P2 and by partition over G25 with 0.1 N AcOH, 1-BuOH, pyridine (11:5:3) to give the products. (In future, purification will be changed to CM-cellulose and partition over G25).

A series of peptide acids comprising <Glu-His-Trp-OH, <Glu-His-Trp-Ser-Tyr-Gly-OH, [Tyr³, Trp⁵]-LRF acid, [Gly^{1a}]-LRF acid [Gly^{2a}]-LRF acid, <Glu-His-Trp-Ser-Tyr-Gly-Leu-Cit-OH, and [des-His², des-Trp³]-LRF acid, were synthesized and found to have low potencies for the release of LH and FSH. None of these analogs showed any clear inhibition of the release of LH and FSH.

The peptide amides, [Tyr³, Trp⁵]-LRF, [Leu², Leu³]-LRF, [Ile²]-LRF, [des-His², des-Leu⁷]-LRF, [des-His², Ala³, D-Ala⁶]-LRF, Gly-Leu-Arg-Pro-Gly-NH₂, D-Ala-Leu-Arg-Pro-Gly-NH₂, [des-His², des-Trp³]-LRF, and [Leu², Leu³, D-Ala⁶]-LRF also had low potencies for LH and FSH release.

The analogs, [Leu², Leu³]-LRF and [Leu², Leu³, D-Ala⁶]-LRF were practically devoid of releasing activity and unequivocally inhibited the release of LH and FSH at 300,000-fold and 30,000-fold dosages respectively. Thus, [Leu², Leu³, D-Ala⁶] LRF is of comparable potency for inhibition as any described inhibitor to date, in the in vitro assay system employed. Interestingly, the desGly¹⁰, ethylamide modification of [Leu², Leu³, D-Ala⁶] LRF does not enhance its LRF antagonist activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of LRF analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesize Nitrogen-Containing Analogs of Prostaglandins
Contractor : University of New Hampshire
Money Allocated : \$27,418 (FY 72); \$14,278 (FY 73)

Objectives: The purpose of this project is to synthesize azaprostaglandin analogs and to test these compounds for antifertility activity.

Major Findings: All of the goals stated in the objectives have been achieved. Seven additional compounds, ethyl N-octanoylprolylglycylglycine, N-octanoyl-3-hydroxyprolyl-5-aminovaleric acid, pentanoylglycylprolyl-5-aminovaleric acid methyl ester, N-hexanoylglycylprolylglycylglycylamide, N-octanoyl-3-hydroxyprolylglycylglycine, N-hexanoylglycylprolylglycineglycine and D, L-N-octanoylprolylglycylglycine, have been synthesized. None of the compounds tested showed prostaglandin-like activity in a postcoital antifertility assay in hamsters.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandin analogs for contraceptive utility.

Proposed Course: Termination. All of the contract objectives have been achieved.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Biological Testing of Thyrotropin Releasing Hormone (TRH) to Determine the Extent and Nature of Antifertility and other Endocrine Activity

Contractor: Abbott Laboratories

Money Allocated: \$83,563 (FY 73)

Objectives: The purpose of this project is to explore the possible efficacy of TRH as a fertility control agent.

Major Findings: The original studies (pre-contract work) on six baboons indicated normal cyclicity with inhibition of ovulation when TRH was administered to baboons orally. These observations remain to be confirmed. No effect of TRH on the ovarian cycle of the baboon was observed at low intramuscular doses up to 0.8 mg/day. However, blockage of ovulation, intramuscularly, could be achieved at doses of 1.2 to 2.4 mg/day. LH suppression, progesterone depression and prolactin elevation were observed.

No effect of TRH on the ovarian cycle in the guinea pig was noted at oral doses up to 5 mg/day (via drinking water). In swine, orally administered TRH (twice daily for full cycle) showed no interference with estrus or cycle length. The results further indicate, that at the doses used (10 and 100 mg daily), in a twice-daily oral regime which was successful with the baboon in earlier work, there is no effect on ovulation in swine.

Significance to Biomedical Research and Programs of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch of developing new contraceptive agents.

Proposed Course: Termination. The major contract objectives have been achieved.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Novel Steroids for Fertility and Population Control

Contractor: University of South Carolina

Money Allocated: \$71,963 (FY 73) for two years

Objectives: The purpose of this project is to synthesize pentacyclic analogs of known effective contraceptive steroids which, due to the unique conformational changes imposed by the introduction of bridged rings in either ring A or ring D, could competitively inhibit progesterone at the receptor site and result in an antiprogestational compound. These compounds will be tested for antifertility activity.

Major Findings: Although a variety of the original synthetic schemes, in addition to several alternate schemes, including Birch reduction of 4b, 5, 6, 10b, 11, 12-hexahydro-2-ethoxy-8-methoxychrysene and cyclization of the tricyclic hydroxy acid in polyphosphoric acid, were tried to produce an important starting material, 18-nor-D-homo-estra-1,3,5(10),13-tetraen-17a-one, none of them had been successful. However, a reliable synthetic method for the synthesis of the B/C cis isomer of 18-nor-D-homo-estra-1,3,5(10),13-tetraen-17a-one methyl ether has been developed by a modification of the Targov procedure. Irradiation of this tetracyclic enone in methylene chloride solution at approximately -70° with continuous bubbling of ethylene through the solution provided the expected [2+2]cycloadduct, 13 β , 14 β -ethano-18-nor-D-homo-estra-1,3,5(10)-trien-17a-one methyl ether in 51% yield. Although the stereochemistry of this cycloadduct was assumed to possess the B/C cis structure, it is essential to determine its correct structure and stereochemistry by x-ray crystallography. If the B/C cis assignment is correct, it is feasible to obtain the desired B/C trans compound by epimerization at C-9. In a similar manner as described above, irradiation of 18-nor-D-homo-estra-1,3,5(10),13(17a)-tetraen-17-one methyl ether provided the expected [2+2]cycloadduct, 13 β , 17a β -ethano-18-nor-D-homo-estra-1,3,5-trien-17-one methyl ether in 85% yield. The stereochemistry of the cycloadduct is assigned on the basis of analogous cycloadditions. This cyclobutyl ketone underwent acid-catalyzed isomerizations, giving a mixture of products from which a new ketone 13 β , 17 β -ethano-18-nor-D-homo-estra-1,3,5-triene-17a-one could be isolated by preparative thin layer chromatography in 32% yield.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis and Testing of Potential Inhibitors of
Luteinizing Hormone Releasing Factor
Contractor: Indiana University Foundation
Money Allocated: \$98,462 (FY 73) for two years

Objectives: The purpose of this project is to synthesize potential inhibitors of luteinizing hormone releasing hormone (LRF) in which unnatural and unusual amino acids are to be substituted for certain natural amino acids in LRF.

Major Findings: The synthesis and purification of three analogs of LRF, viz, [pyro-L-~~α~~-aminoadipic]¹ LRF, [~~δ~~-N-isopropyl-L-ornithine]⁸ LRF and [L-~~β~~-naphthylalanine]³ LRF, have been completed utilizing a combination of classical and solid phase methods. These compounds are currently being evaluated as potential inhibitors of LRF.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of potential inhibitors of LRF for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of 12 α -Methyl Prostaglandins Related to
PGF₂ α
Contractor: University of Pittsburgh
Money Allocated: \$72,736 (FY 73) for two years

Objectives: The purpose of this project is to synthesize 12 α -methyl PGF₂ α , and 12 α -methyl-15 β -methyl-PGF₂ α and to test these compounds for antifertility activity. The extra methyl group may serve to block one of the pathways of elimination of the 11 α -hydroxy group in the prostaglandins. This novel structural modifications would be important to study insofar as structure-activity relationships of the prostaglandins are concerned.

Major Findings: A total synthesis of 12 α -methyl PGF₂ α has been initiated starting from the optically active hydrindenedione which was, in turn, prepared reproducibly in 80-84% yield by asymmetric cyclization of 2-methyl-2-(3'-oxobutyl)-1,3-cyclopentanedione. Attempts to synthesize 12 α -methyl PGF₂ α from this compound have resulted in failure thus far. The synthesis of the optically active phosphonium salt required for the construction of the C-8 side chain has been completed. Two intermediates, 1-cyclopentyloct-1-yne-3-ol and 1-cyclopentyloct-1-ene-3-ol have been submitted for testing.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandins and related analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Novel Compounds of Potential Anti-Fertility Activity
Contractor: University of California
Money Allocated: \$23,994 \$9,231 (FY 73)

Objectives: The purpose of this project is to synthesize cis- and trans-4,6-cyclopropane analogs of certain contraceptive steroids which, due to the unique geometrical conformations imposed by the introduction of the cyclopropane ring, could competitively inhibit progesterone at the receptor site and result in an antiprogestational compound. These compounds will be tested for antifertility activity.

Major Findings: 5 β -4,6-Cyclopropano-3,20-dione was inactive in the Clauberg assay and exhibited no significant binding activity relative to progesterone. The corresponding 5 α (trans) analog, interestingly, displayed 30-40% binding affinity relative to progesterone, but was inactive in the Clauberg, anti-Clauberg and postcoital antifertility assays. 5 β -4,6-Cyclopropanoandrostone-17 β -ol-3-one acetate and the corresponding 19-nor-3,17-dione displayed neither androgenic nor antiandrogenic activity. The 5 β -4,6-cyclopropano analog of norethindrone was synthesized and failed to show activity in a Clauberg and a postcoital antifertility assay. Thus, none of the compounds tested in this series showed interesting endocrinological properties.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: Termination.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Novel Steroids as Potential Antifertility Agents
Contractor : The John C. Sheehan Institute for Research, Inc.
Money Allocated : \$27,072 (FY 73) \$32,510 (FY 74)

Objectives: The purpose of this project is to synthesize a new class of steroidal compounds with a cyclopentane ring fused at C4 and C6 as potential antifertility agents.

Major Findings: The synthetic scheme proceeded satisfactorily up until the cyclization of 5-(2-(1,3-diketo-2-methyl-2-cyclopentanly)ethylidene)-8-methoxy-2a,3,4,5-tetrahydroacenaphthene using p-toluenesulfonic acid to give 4,6-cyclopentano-3-methoxyestra-1,3,5(10), 8,14-pentaen-17-one. At this point highly colored by-products and stereoisomers were obtained. It has been subsequently found that the use of boron trifluoride etherate gives a very considerable increase in the yield of the cyclization product, and the purification of this product is now simplified. The spectral data also suggested that a mixture of double bond isomers may be formed in the catalytic hydrogenation of 4,6-cyclopentano-3-methoxyestra-1,3,5(10)8, 14-pentaen-17 - ol. This problem could be circumvented by choice of reaction conditions, e.g., using 5-10% palladium on carbon in benzene.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Ring-A Bi- and Tricyclo Steroids with Potential Antifertility Activity
Contractor: Stanford Research Institute
Money Allocated: \$55,543 (FY 73), \$35,201 (FY 74)

Objectives: The purpose of this project is to synthesize steroids containing bridged bi- and tricyclo systems in ring-A, which will result in alteration of the binding of the steroid to the receptor protein. These compounds will be tested for antifertility activity.

Major Findings: Four compounds, two in the tricyclosteroid series (ring-A tricyclo-17 α -methyltestosterone and ring-A tricycloprogesterone) and two intermediates in the 1 α -vinylsteroid series (1 α -vinyltestosterone and 1 α -vinylprogesterone) have been synthesized. The tricyclotestosterone analog showed neither androgenic nor antiandrogenic activity. 1 α -Vinyltestosterone did not display androgenic activity. Several other tricyclo and vinyl steroids are being prepared. The synthesis of ring-A bicyclo [2.2.0] hexane derivatives will be pursued.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Novel Steroids as Potential Antifertility Agents
Contractor : The John C. Sheehan Institute for Research, Inc.
Money Allocated : \$27,072 (FY 73) \$32,510 (FY 74)

Objectives: The purpose of this project is to synthesize a new class of steroidal compounds with a cyclopentane ring fused at C4 and C6 as potential antifertility agents.

Major Findings: The synthetic scheme proceeded satisfactorily up until the cyclization of 5-(2-(1,3-diketo-2-methyl-2-cyclopentanyl)ethylidene)-8-methoxy-2a,3,4,5-tetrahydroacenaphthene using p-toluenesulfonic acid to give 4,6-cyclopentano-3-methoxyestra-1,3,5(10), 8,14-pentaen-17-one. At this point highly colored by-products and stereoisomers were obtained. It has been subsequently found that the use of boron trifluoride etherate gives a very considerable increase in the yield of the cyclization product, and the purification of this product is now simplified. The spectral data also suggested that a mixture of double bond isomers may be formed in the catalytic hydrogenation of 4,6-cyclopentano-3-methoxyestra-1,3,5(10)8, 14-pentaen-17-ol. This problem could be circumvented by choice of reaction conditions, e.g., using 5-10% palladium on carbon in benzene.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Ring-A Bi- and Tricyclo Steroids with Potential Antifertility Activity
Contractor: Stanford Research Institute
Money Allocated: \$55,543 (FY 73), \$35,201 (FY 74)

Objectives: The purpose of this project is to synthesize steroids containing bridged bi- and tricyclo systems in ring-A, which will result in alteration of the binding of the steroid to the receptor protein. These compounds will be tested for antifertility activity.

Major Findings: Four compounds, two in the tricyclosteroid series (ring-A tricyclo-17 α -methyltestosterone and ring-A tricycloprogesterone) and two intermediates in the 1 α -vinylsteroid series (1 α -vinyltestosterone and 1 α -vinylprogesterone) have been synthesized. The tricyclotestosterone analog showed neither androgenic nor antiandrogenic activity. 1 α -Vinyltestosterone did not display androgenic activity. Several other tricyclo and vinyl steroids are being prepared. The synthesis of ring-A bicyclo [2.2.0] hexane derivatives will be pursued.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Study of Compounds for Inhibition of Spermatogenesis
Contractor: The Childrens Hospital of Philadelphia
Money Allocated: \$23,843 (FY 73) \$35,000 (FY 74)

Objectives: Certain steroidal compounds have been found, in vitro, to selectively block testicular conversion of prenenolone to testosterone, but not adrenal conversion to corticosterone. It is the objective of this contract to study these compounds in vivo for testicular pregnenolone inhibition and subsequent blockage of spermatogenesis.

Major Findings: The results obtained to date indicate that 5 β -cyano-17 α -ethynyl-19-nor-dihydrotestosterone produces marked effects on the adult male rat when administered in daily doses over a two week period. The highly significant prevention of body weight gain, marked depression in target organ weights, and adrenal enlargement can be interpreted as an inhibition of testicular steroidogenic function. This interpretation is confirmed by the finding that the 2 week treatment period results in a 95% depression of the testicular testosterone content.

The present results also show that the testes of male rats treated with 5 β -cyano-17 α -ethynyl-19-nor dihydrotestosterone have a significant in vivo depression of the conversion of pregnenolone, progesterone, or DHA to testosterone. The predominant sites of the inhibition produced by 5 β -cyano-17 α -ethynyl-19-nor-dihydrotestosterone is at the level of the conversion of C₂₁-precursors to C₁₉-androgens by 17 α -hydroxylase, C₁₇₋₂₀ lyase, and 17-ketoreductase.

The present results also indicate, as had been previously anticipated, that adrenal conversion of pregnenolone and DHA are virtually unaffected by 5 β -cyano-17 α -ethynyl-19-nor-dihydrotestosterone and 5 α -cyano-17 α -(2-methallyl)-19-nor-dihydrotestosterone with the possible exception that activity of 5 α -reductase in the adrenal may have been depressed, thus allowing for a potentially larger production of adrenal corticoids. 5 α -cyano-17 α -(2-methallyl)-19-nor-dihydrotestosterone appears to block testicular biosynthesis of testosterone, with a 54% drop in testicular testosterone content.

An effect of 5 β -cyano-17 α -ethynyl-19-nor-dihydrotestosterone on spermatogenesis could not be shown in the present 2 week test period. It would be indeed interesting if in a longer test period of 6 weeks no effect either on spermatogenesis or male fertility were found in view of the drastic reduction in testicular testosterone content. Continuation studies are planned.

Δ^2 -3-cyano-17 β -hydroxy-5 α -androstane acetate also produces marked effects on target organ weights without affecting weight gain, adrenal weight, or liver size. Biochemical studies are in progress and should confirm a testosterone depletion. The fact that fertility of the males treated with this compound

is not affected may possibly be attributable to the relatively low dose employed. The lower degree of effectiveness of this agent in reducing target organ weights is consistent with this interpretation.

Significance to Biomedical Research and Programs of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch of developing new contraceptive agents for use of the male population.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Steroidal Compounds Possessing a Bridged Bicyclic A-ring with Potential Antifertility Activity
Contractor: Research Triangle Institute
Money Allocated: \$26,860 (FY 73) \$26,683 (FY 74)

Objectives: The purpose of this project is to synthesize bicyclo [2.2.0] hexene derivatives of steroids possessing the zero bridge between carbon atoms 1 and 4 and test these compounds for antifertility activity.

Major Findings: A key intermediate for the synthesis of A-ring bicyclo [2.2.0] hexyl steroids, vinylene carbonate, has been obtained in poor yield. At present the photolytic cycloaddition of acetylene to the vinylene carbonate is under investigation. Low yields, which have required the preparation of additional quantities of starting compound have slowed the overall synthetic time table.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract: Synthesis and Testing of Irreversible Inhibitors of Uterine Progesterone Binding Factors. Synthesis and Testing of Irreversible Inhibitors of Uterine Estradiol Binding Factors.

Contractor: Research Triangle Institute

Money Allocated: \$64,490 (FY 73) \$68,490 (FY 74)

Objectives: The objective of this project is to synthesize and test a variety of derivatives of estradiol (first year) and progesterone (second year) which would have the ability to bind to estrogen and progesterone receptor sites respectively in the target tissue. These derivatives would contain bridging groups terminating in an arylsulfonyl fluoride. This reactive group could possibly form a covalent linkage to the receptor and thus, would elicit either a hormonal or antihormonal condition in the target tissue. Such compounds may be useful as contraceptive agents.

Major Findings: Methodology has been set up for routinely determining the competitive binding of progestins and estrogens to their respective receptors in vitro, and for measuring the blockage of uptake of the hormones by the uterus in vivo. A method for discriminating between reversible and irreversible binding is being established. Substituted estradiol derivatives have been prepared, all of which bear a 7α or a 17α -(2-carbamidoethylthio) group. Further substitution on the carbamido group terminates eventually in a fluorosulfonylaryl moiety. These compounds were chemically characterized and tested for binding, as were a series of 11α -substituted progesterone derivatives based on the hemisuccinate of 11α -hydroxyprogesterone and a series of 7α -(2-carbamidoethylthio) progestosterone derivatives. The binding affinity of these compounds is too low to make them promising candidates for irreversible binding inhibitors. The estradiol derivatives showed no estrogenic or antiestrogenic activity in rats.

Significance to Biomedical Research and Programs of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to develop new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
JULY 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Novel Steroidal Gestagens
Contractor: University of Illinois at Chicago Circle
Money Allocated: \$30,033 (FY 73), \$11,500 (FY 74)

Objectives: The purpose of this project is to synthesize ring B bicyclo [2.2.0] hexene derivatives of progesterone and ring A/B cyclopene derivatives of progesterone, which may have the ability to compete with progesterone for the receptor protein and thereby act as antiprogesterone agents.

Major Findings: The photolysis of $9\alpha, 10\alpha$ -pregna-5,7-dien- 3β -ol-20-one acetate and $9\beta, 10\beta$ -pregna-5,7-dien- 3β -ol-20-one acetate without protection of the C-20 carbonyl group did not give the expected ring B-bicyclo [2.2.0] hexene. Presumably, the pentacyclic alcohols were formed in analogy to a well known reaction in the literature. Obviously, this difficulty can be circumvented by protection of the C-20 carbonyl group by formation of an ethylene ketal and synthesis of this compound has been achieved as far as the 5,7-protected diene.

Very recently a reliable synthetic method for $9\alpha, 10\alpha$ -pregna-ring B-bicyclo [2.2.0]hexene- 3β -ol-20-one acetate has been developed by reduction of the C-20 carbonyl group of $9\alpha, 10\alpha$ -pregna-5,7-dien- 3β -ol-20-one acetate to a mixture of the corresponding alcohol, irradiation, and Collins oxidation.

Conversion of the 19-hydroxypregnenolone acetate into 3β -acetoxy- $5\beta, 19$ -cyclopregna-6-en-20-one was successfully carried out. However, several attempts to introduce the 8,9-double bond into the former compound failed. The solvolysis of 19-mesyloxy-5,7-cholestadien- 3β -ol acetate as a model system gave the unexpected $5\beta, 19$ -cyclo-6,8(14)-cholestadiene- 3β -ol acetate but not the desired $5\beta, 19$ -cyclopregna-6,8(9)-diene-20-one. The latter compound will be synthesized from solvolysis of the C_{19} -mesyloxy- $\Delta^{5,7}$ -diene in the cis- $9\beta, 10\beta$ series. $5\beta, 19$ -Cyclo-6-pregnen-3,20-dione was synthesized and was found to be inactive in the Clauberg assay at 2.0 mg., s.c.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesis of Novel Steroids with Potential Antifertility Activity
Contractor: Temple University
Money Allocated: \$26,990 (FY 73), \$6,455 (FY 74)

Objectives: The purpose of this project is to synthesize novel spiro steroids, ring A bicyclo [3.2.0] heptanes, and cyclopropyl steroids. These compounds will be tested for antifertility activity.

Major Findings: 17β -Acetoxy-4-keto- $\Delta^{1(10),2,5}$ -trien-A-homo-steroid has been synthesized in 30% yield according to the known procedure. Nevertheless, direct irradiation of this tropolone compound in benzene, ether or methanol as a solvent with a medium-pressure mercury arc (Hanovia 450W) through a pyrex filter generated a complex mixture, whose nuclear magnetic resonance spectrum indicated the presence of the target compound, 1,4a-cyclo-A-homosteroid. However, final verification must await isolation, purification, and spectral analysis. The synthesis of 4-keto- $\Delta^{5,10}$ -A-homosteroid has been accomplished by desulfurization of $5\beta,19$ -cyclo- Δ^3 androsten- $[3,4-b]$ dithiane-17-one followed by solvolysis in aqueous acid and oxidation. Ethynylation of 4-hydroxy- $\Delta^{5,10}$ -A-homosteroid-17-one, and $5\beta,19$ -cyclo- Δ^3 androsten- $[3,4-b]$ dithiane-17-one gave the corresponding 17α -ethynyl compounds. Deconjugation of 17β -acetoxyestra-4,9(10)-dien-3-one and photolysis of the resulting 17β -acetoxyestra-5(10),9(11)-dien-3-one gave a novel spiro compound, (10R)- 17β -acetoxy-3,10-cyclo-3,4-seco- 10α -estra-4,9(11)dien-1-one. The conjugated cyclopropyl ketone, 17α -ethynyl- 17β -hydroxy- $5\alpha,19$ -cyclo-A-norandrostan-3-one has been synthesized by photolysis of norethynodrel.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Non-Steroidal Nitroheterocycles with Potential Antifertility Activity
Contractor: Rensselaer Polytechnic Institute
Money Allocated: \$32,458 (FY 73), \$8,600 (FY 74)

Objectives: The purpose of this project is to synthesize several series of five-membered hetrocycles containing electron withdrawing substituents for evaluation as possible antifertility agents.

Major Findings: Utilizing the "masked" 1,3-dipole present in the oxazolium mesoionic ring system, reaction with 1-bromo-1-nitro-2-phenylethylene as the dipolarophile gave 1-methyl-2,4,5-triphenyl-3-nitropyrrole as the major product and 1-methyl-2,4,5-triphenyl-3-bromopyrrole as the minor product. This reaction does not appear to be as general as proposed. In order to overcome this obstacle to the nitro-substituted pyrazoles, an alternative 1,3-dipolar cycloaddition of diazomethane with the 1-bromo-1-nitro-2-phenylethylene was employed to obtain 3-nitro-4-phenyl-1H-pyrazole. Subsequent alkylation of the nitropyrazole under standard conditions resulted in the formation of several alkylated products such as 1-methyl-3-nitro-4-phenylpyrazole, and 1-isoamyl-3-nitro-4-phenylpyrazole. Alkylation of the commercially available 3-methyl-4-nitro-1H-pyrazole with N, N-diethylaminocarbonylmethyl chloride under standard conditions afforded 1-(N,N-diethylaminocarbonylmethyl)-3-methyl-4-nitropyrazole. Evaluation of these compounds in the male antifertility screen is in progress, but thus far none of the nitropyrazoles tested have shown biological activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of non-steroidal heterocycles for male contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis and Testing of Antifertility Compounds
Contractor: Midwest Research Institute
Money Allocated: \$31,634 (FY 74)

Objectives: The purpose of this project is to synthesize a group of spiro-derivatives of the known contraceptive phenylcyclohexenecarboxylic acids exemplified by Fenestrel and Carbestrol. It is hoped that the rapid destruction of Fenestrel and Carbestrol in the human may be prevented by blocking aromatization of the cyclohexene ring through introduction of the spiro derivatives. Such components may be useful as contraceptive agents.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of non-steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Antifertility Compounds New and Novel
 Compounds Structurally Related to Steroids.
Contractor: Oklahoma State University
Money Allocated: \$18,000 (FY 74)

Objectives: The purpose of this project is to synthesize analogs of contraceptive steroids in which the B/C rings have been separated by a four-membered ring. These compounds will be tested for antifertility activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis and Testing of Antifertility Compounds
Contractor: IIT Research Institute
Money Allocated: \$14,888 (FY 74)

Objectives: The purpose of this project is to synthesize base-substituted steroidal hormonal agents and sparingly soluble complexes thereof utilizing a polyvalent metal ion, such as Zn^{++} or Al^{+++} , and a polybasic acid, initially tannic acid. These metal complexes will serve as long-acting depot preparations for intramuscular injection. The base-substituted steroids will also be tested for antifertility activity and other hormonal effects.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Luteolytic Prostanoids.
Contractor: University of Washington
Money Allocated: \$55,316 (FY 74)

Objectives: The purpose of this project is to synthesize prostaglandin analogs as potential luteolytic agents, and test these compounds for anti-fertility activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandins and related analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesis and Testing of Antiprogestational Agents
Derived from 7(8 \rightarrow 11 α)Abeo-steroids
Contractor: Research Triangle Institute
Money Allocated: \$121,576 (FY 74) for two years

Objectives: The purpose of this project is to synthesize 7(8 \rightarrow 11 α)abeo-steroids, 1, 11-methano and 1,11-ethano bridged steroids, which may exhibit antiprogestational properties. These compounds will be tested for antifertility activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesis and Testing of Prostaglandin Synthetase Inhibitors with Potential Antifertility Activity.
Contractor: Stanford Research Institute
Money Allocated: \$99,710 (FY 74)

Objectives: The purpose of this project is to synthesize potential prostaglandin synthetase inhibitors as potential antifertility agents, specifically as ovulation inhibitors. The structures to be synthesized are tri-unsaturated C-20 acid analogs of the prostaglandin precursor γ -homolinolenic acid, carrying alkyl groups in strategic positions so as to interfere with or prevent the formation of known intermediates in the prostaglandin biosynthesis.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandins and related analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesis and Testing of Some Novel 11-Oxa-16-Substituted Corticosteroids as Potential Antifertility Agents.

Contractor: Southwest Foundation for Research and Education

Money Allocated: \$102,028 (FY 74)

Objectives: The purpose of this project is to synthesize a variety of 11-oxa-16-substituted corticosteroids as potential antifertility agents unaccompanied by corticoid properties although the proposed compounds are structurally related to the corticosteroids.

Significance to Biomedical Research and Program of the Institute: The work undertaken in the project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of Seco and Nor Analogs of Norethindrone
and Progesterone
Contractor : Oregon Graduate Center
Money Allocated : \$33,921 (FY 74)

Objectives: The purpose of this project is to synthesize certain Seco and Nor analogs of norethindrone and progesterone as antifertility agents. It is hoped that the rotational freedom of the A ring will lead to slower enzymatic reduction of the unsaturated ketone system and hopefully lead to increased biological activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of unusual skeletal modifications of steroids for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesis of 15-Phosphasteroids for Antifertility
Screening
Contractor: Duke University
Money Allocated: \$18,850 (FY 74)

Objectives: The purpose of this project is to synthesize some isosteres of progesterone and norethindrone in which C-15 is replaced by a phosphorous atom. The key reaction for construction of the D-ring involves a cycloaddition of methylphosphorous dichloride with the appropriate diene. Subsequent reactions for conversion of the tricyclic phosphorus compound into the steroid analogs follow well established routes. These compounds will be tested for antifertility activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Ring-C Aromatic Steroids as Potential Anti-fertility Agents.
Contractor: The John C. Sheehan Institute for Research, Inc.
Money Allocated: \$72,871 (FY 74) for two years.

Objectives: The purpose of this project is to synthesize a series of ring-C aromatic steroids by total synthesis as novel antiprogestational agents. These compounds will be tested for antifertility activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Biological Testing Facility for the Evaluation of
New Antifertility Drugs and Devices

Contractor: Mason Research Institute

Money Allocated: \$844,057 (FY 72) 30 months, \$116,436 (FY 73)

Objectives: The objective of this contract program is the establishment of a biological testing facility capable of broad spectrum evaluation of new drugs and devices and rapid exploitation of potential leads in the control of fertility. Sources of compounds for testing include contract synthetic programs supported by the CDB and numerous private, public and governmental laboratories throughout the world. The staff of the CDB reviews all compounds submitted for evaluation to determine the precise manner in which each drug will be studied.

Major Findings: Almost 200 compounds have been tested for antifertility and classical endocrine or endocrine antagonist activities. Several potential leads have been identified and a significant separation of antifertility and endocrine activities has been demonstrated for several members of at least one chemical class of compounds. Two members of this series are being studied in the monkey to confirm the separation of estrogenic and postcoital antifertility activities in a primate species.

Baseline studies on 16 reference standards have been completed and are being utilized in the design of assays for the potency of experimental compounds.

The contractor is cooperating with CDB staff in the development of new and more sophisticated test procedures on a continuing basis. These include modifications of postcoital antifertility and antispermatogenic tests and the establishment of an in vitro assay for oxytocic activity using the hamster uterus.

Data generated by the testing facility is communicated to CDB staff on a weekly basis and is then rapidly disseminated to individual chemists in our contract synthetic program. This input at the drug synthesis level permits an interaction between chemist and biologist which provides optimal direction for subsequent structural modifications.

Significance to Biomedical Research and Program of the Institute: The heart of any drug development program is the rapid identification of biological activity and exploitation of promising leads. The ability of the CDB to evaluate compounds and devices in a uniform manner is of great importance to our contract synthetic program and the stated goals of the Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Implantable Bio-Absorbable Capsules as Slow-Release
Contraceptive Drug Delivery Systems
Contractor: Abcor, Inc.
Money Allocated: \$254,856 (FY 73 for two years)

Objectives: The contractor proposed to formulate a biodegradable sustained release drug delivery system consisting of microcapsules containing progesterone. The system will be evaluated for its ability to alter the characteristics of cervical mucus and thus produce its contraceptive effect. The microcapsules will be injected directly into cervical tissue and it is hoped that such a delivery system will not interfere with menstrual cyclicality and avoid other systemic side effects. It can also be utilized for the delivery of other contraceptive drugs where sustained long term delivery of the drug is desired.

Major Findings: Several bio-absorbable polymers were prepared from polylactide and polyglycolide for the microcapsule wall of the progesterone delivery system. Their composition and molecular weights were selected to reflect a spectrum of biodegradation rates. Previous work with the polymers has shown degradation rate to be directly related to reduced specific viscosity (η) which in turn may be correlated with molecular weight. Homopolymers of L (-) lactide were prepared with specific viscosity between 0.34 and 4.23 dl/g. The latter figure, to our knowledge, is the highest value ever reported for the polymer, and is equivalent to a molecular weight of about 300,000. This material should degrade very slowly, to provide a good wall for the desired long term hormone delivery microcapsule. Two homopolymers were also prepared from DL-lactide. Their specific viscosity is 0.69 and 1.79 dl/g. They have different solubility characteristics from the L(-) polymer which may enhance their microencapsulation by coacervation. For fast biodegradation a series of lactide/glycolide copolymers (55.5%/45.5%) were additionally prepared. Their specific viscosity ranges between 0.27 and 0.74 dl/g.

A significant effort was devoted to the development of progesterone microcapsules with the above polymers. Two different microencapsulation techniques, coacervation and coagulation, were examined in depth. Microencapsulation by coacervation depends on phase separation in polymer solutions. Efforts with coacervation technique were hampered by the similarity between the solubility characteristics of the polylactides and progesterone. Both are soluble in the same class of solvents. An extensive investigation of numerous solvents resulted in the discovery of two potential solvents, triethylcitrate (TEC) and triacetin.

Significance to Biomedical Research and Program of the Institute: Development of new drug delivery systems for contraceptive drugs is one of the goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development and Evaluation of a Biodegradable Drug Delivery System
Contractor: Arthur D. Little, Inc.
Money Allocated: \$60,900 (FY 74)

Objectives: This program is directed toward the development of an implantable biodegradable delivery system for the administration of fertility regulating agents. The proposed delivery system consists of a polymeric matrix containing a physically admixed drug, where synthetic polypeptides (poly- α -amino acids are used for the matrix.

Major Findings: Recent findings support the basic feasibility of the use of synthetic polypeptides as biodegradable drug delivery vehicles. The rates of biodegradation achieved to date, however, have been either too rapid or too slow to provide adequate drug release for the desired 90-120 days. For example, a copolymer containing equal mole percents of glutamic acid and leucine was found to be absorbed in approximately 24 hours. On the other hand, a copolymer consisting of 20 mole percent glutamic acid and 80 mole percent leucine (20/80) showed little evidence of biodegradation in 90 days. The in vitro and in vivo release of progesterone from 20/80 copolymer was studied. As anticipated, the release of progesterone into the enzyme-free in vitro medium was characteristic of a simple diffusional mechanism occurring in a relatively rigid polymer. The calculated polypeptide permeability to progesterone was approximately 1×10^{-13} cm²/sec.

When 20/80 films containing 9.09% (W/W) progesterone were subcutaneously implanted in the backs of rats, a nearly constant rate of release in the range of 0.1 to 1.0 $\mu\text{g}/(\text{day}) (\text{cm}^2)$ was obtained, depending upon film geometry. This low rate of release may be due to the slow rate of enzymatic hydrolysis of the polypeptide copolymer and the limited fluid exchange taking place at the implant site.

Significance to Biomedical Research and Program of the Institute: Development of drug delivery systems for contraceptive drugs is one of the stated goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Study of Biodegradable Polymers for the Sustained Delivery of Contraceptive Drugs
Contractor: Research Triangle Institute
Money Allocated: \$299,994 (FY 73) for two years

Objectives: It is proposed to develop a biodegradable polymer drug delivery system for male and female fertility regulation. The release of the appropriate drug will be dependent on the rate of polymer by hydrolysis and will be independent of diffusion rate.

The specific polymers which will be evaluated are the aliphatic polyesters. They will be selected on basis of tissue compatibility, biodegradability, synthetic availability, and structured variability. The performance of the delivery system will be evaluated by a number of different tests.

Major Findings: Homopolymers of dl- and l-lactic acid, dl-2-hydroxy-2-methylbutyric acid, δ -hydroxyvaleric acid and ϵ -hydroxycaproic acid, and copolymers of glycolic acid-dl-lactic acid, glycolic acid- ϵ -hydroxycaproic acid and dl-lactic acid- ϵ -hydroxycaproic acid have been synthesized and characterized.

Films of poly(dl-lactic acid) and poly(ϵ -hydroxycaproic acid) containing varying amounts (10-30% w/w) of steroidal contraceptive drugs (progesterone, norgestrel, testosterone and testosterone propionate) have been prepared. The rates of drug release from these films have been measured in vitro and in vivo (rabbit), and the dependence of the release rates on polymer molecular weight and thickness; drug structure and drug load, has been determined. The diffusion coefficients of steroidal drugs in poly(dl-lactic acid) and poly(ϵ -hydroxycaproic acid) have been measured. The results obtained indicate that poly(ϵ -hydroxycaproic acid) is comparable to Silastic in its ability to permit rapid diffusion of steroids, whereas poly(DL-lactic acid) is much less permeable.

Preliminary studies of the biodegradation of these polymers in rabbit over a 4-month period indicate the rate of biodegradation is dependent on both the molecular weight and chemical structure of the polymer. Both poly(dl-lactic acid) and poly(ϵ -hydroxycaproic acid) degraded slowly (<10%), but copolymers were substantially degraded. The rate of biodegradation decreased with increasing molecular weight.

Significance to Biomedical Research and Program of the Institute: Development of drug delivery systems for contraceptive drugs is one of the stated goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development and Testing of an Implantable Contraceptive Delivery System

Contractor: Dynatech R/D Company

Money Allocated: \$245,972 (FY 73 for 2 years)

Objectives: It is proposed to develop and test new implantable drug delivery systems based on synthetic polymers which slowly release the incorporated drug. The implantable material will consist of tissue compatible hydrolyzable polymers material such as polyglycolic acid, polylactic acid, as copolymers in which the synthetic progestin d-norgestrel will be dispersed. As the polymers undergo slow hydrolysis, the physically entrapped drug will be released. At the end of a planned period of time both the drug and polymer material are absorbed by the surrounding tissues.

Major Findings: It has been clearly demonstrated that glycolide-lactide copolymers with 25% or more glycolide absorb far too rapidly for an implant designed to last 6 months. Sustained release of d-Norgestrel for over 3 months has been demonstrated for homopolymers of dl-lactic acid and for L+ lactic acid.

Dogs have been implanted with rods made from a dl-lactic acid homopolymer and a 10/90 glycolide dl-lactide copolymer. Preliminary results indicate a longer release rate for these implants than for the 25 glycolide 75 dl-lactide copolymer previously tested.

Tests on rabbits using a 50/50 glycolide lactide copolymer show that the polymer is virtually all absorbed in about 5 weeks. No sign of inflammation or tissue reaction was noted. Drug containing polymer was also implanted in rabbits at MRI. These implants released a negligible amount of ¹⁴C tagged steroid into the urine until the polymer matrix had been well absorbed which is quite different than the behavior of the 25/75 copolymer implant containing drug implanted in the dog where the ¹⁴C label was found in the dogs urine almost immediately. On the other hand, considerable ¹⁴C radioactivity was found in the rabbits feces after 3 weeks; the only time the feces were collected showing that the rabbit excretes much more of the d-Norgestrel in the feces than in the urine. This indicates that when rabbits are the test animal, both feces and urine must be monitored and that probably considerable d-Norgestrel was being released all during the 5 weeks test period just as it was in the dog. The implant in the dog was in the thigh area near an active muscle. The implants in the rabbit were in the scapular region which is underlaid by thick bands of fat.

Significance to Biomedical Research and Program of the Institute: The development of improved drug delivery systems for contraceptive drugs is one of the stated goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Intrauterine Release of Estriol for Contraception
Contractor: Michael Reese Hospital
Money Allocated: \$133,233 (FY 74)

Objectives: The objective of this contract is to investigate the local contraceptive action of the hormone estriol. The estriol will be delivered to the uterine cavity by means of an intrauterine device which will be composed of polymeric membrane which can control drug release. The devices will be evaluated initially in rabbits and subsequently in baboons. If local action of the released estriol can be demonstrated a clinical trial will be initiated. The release of small quantities of estriol directly into the uterine cavity should circumvent undesirable systemic effects.

Major Findings: Uterine horns bearing the estriol releasing IUD's were essentially devoid of implants. Only one out of twelve horns had implants and their number was lower than in the contralateral horn. Contralateral horns of all rabbits had implants. With the release rate of 1.2 μ g of estriol per day there appears to be no effect on fertility in the contralateral horn bearing the placebo IUD.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive methods is a stated goal of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved IUD's.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Polyelectrolyte Delivery Systems for Chemical
Cauterization and Sustained Release
Contractor: Columbia University
Money Allocated: \$86,000 (FY 74)

Objectives: The central objective of this project is the development of bio-absorbable controlled release devices for dispensing biologically active substances in vivo. The immediate goal is the design of such devices to achieve chemical cauterization of the oviduct. The specific tasks to be met include:

1. Prepare a drug delivery system for silver ions based upon polyelectrolytes and a biodegradable matrix.
2. Study in vitro rate of Ag⁺ delivery from the rods as a function of systems composition and time.
3. Implant prototype systems initially in rabbits and subsequently in monkeys and study: (a) the degree of occlusion: (b) ingrowth of fibroblasts into the matrix: (c) the rate of degradation of the system, and (d) what happens to residual products.

Major Findings: Several types of oviduct inserts, based principally on the use of alginates as the matrix carrier for silver compounds, have been prepared. Analytical procedures were adapted for the determination of these agents and diffusion experiments were carried out in several kinds of media to establish the compatibility of insert design with in vivo release rate requirements. The optimum shape of the release rate mode aimed at is given by a square-wave, with the action time limited to 24 hours.

Several devices were prepared, one of which approached the ideal delivery mode according to in vitro tests.

Significance to Biomedical Research and Program of the Institute: Development of technology for the non-surgical occlusion of oviducts for contraceptive purposes is a stated goal of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Study of Intrauterine Microcapsules as a Prolonged
 Drug Release Form
Contractor: Battelle Memorial Institute
Money Allocated: \$16,200 (FY 71), \$86,240 (FY 72)

Objectives: The purpose of this project is to investigate the possibility of using a microencapsulated drug dose form as a contraceptive method. The feasibility of this system will depend largely on the distribution of various sizes of microcapsules within the female reproductive tract. This drug delivery system could involve the microencapsulation of progestagens, spermicidal agents or cervical mucus thickeners.

Major Findings: The contractor has continued to study the distribution of various sizes of microcapsules and microspheres within the reproductive tract of rabbits following both intrauterine and vaginal placement. Repeat studies indicated no migration of the $115 \pm 10 \mu$ diameter tracer microcapsules from the uterus into the fallopian tubes as had been suggested earlier. However the number of $15 \pm 5 \mu$, 50 ± 10 or $115 \pm 10 \mu$ diameter microcapsules inserted and remaining in the rabbit uterus upon necropsy indicates a large loss via the cervix and vagina over the first 24 to 72 hours after instillation. Studies with oophorectomized, intact rabbits in constant estrus and mated animals indicated little difference in the migration pattern of microcapsules under those conditions.

In an effort to increase the specific activity of the smallest microcapsules to facilitate more accurate tracing of their distribution, the contractors are exploring means of attaching the radiolabel (^{131}I) to the capsule surface by exchanging it for bromine deposited there by the cyanogen bromide reaction. This method appears successful at this time and seems to have no effect on progesterone release rates.

Work is also continuing on the development of a biodegradable capsule composed of d,l-poly lactide. Success has been achieved in this area although the rate of biodegradation remains to be determined. Progesterone has been encapsulated and release rates are in progress.

The rat is being used for additional studies on transcervical microcapsule migration following initial placement in the vagina and stump-tail monkeys will be employed for this type of investigation shortly.

Significance to Biomedical Research and Program of the Institute: The development of a suitable microcapsule system for sustained or targeted drug delivery of antifertility agents in the female would make a substantial contribution to contraceptive methodology and is directly relevant to the purposes of CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptive methods and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Study of the Factors that Control Diffusion Rates in Controlled-Release Contraceptive Systems.
Contractor: Southern Research Institute
Money Allocated: \$84,101 (FY 72 - 15 months)

Objectives: This proposal aims to quantify individual factors affecting diffusion rate of steroidal contraceptive drugs through polymeric materials (membranes).

Major Findings: Structural and chemical features of steroids influence their diffusional properties in what seems to be a predictable manner.

The morphological characteristics of siloxane polymers influence the diffusional characteristics of steroids in the polymers. Because of higher interchain attractions and lower flexibility of PSSS chains, steroids have much lower values of D in PSSS than in PDMS. Thus, even though the steroids are more soluble in PSSS than in PDMS, the diffusive fluxes are lower through PSSS than through PDMS.

High values of the partition coefficient, K, of a steroid between a polymer and an external phase cause the diffusional resistances of the boundary layers in the external phase to be high relative to the boundary layers in systems with lower values of K. This finding is important to designers of controlled-release contraceptive devices because the resistances of boundary layers are in series with the diffusional resistance of the membrane, and must be taken into account.

Estrone appeared to be molecularly associated in PDMS. The presence of fumed-silica filler in PDMS decreases diffusional flux. The particles of silica increase the tortuosity of the membrane, decrease the volume fraction of polymer present, and appear to retard diffusion by adsorption of steroid.

The diffusional characteristics of progesterone in peroxide-cured PDMS (Silastic sheet stock) differ considerably from those in room-temperature vulcanizing PDMS (Silastic 382). Differences in the molecular weights and crosslinking functionality of the prepolymers used for the two types of PDMS are probably responsible for the differences in diffusional characteristics. Careful characterization and descriptions of the polymeric materials used in studies of controlled-release systems are necessary for the results to be meaningful.

The diffusive flux of progesterone through PDMS was much higher when it was suspended in polytrifluoropropylmethylsiloxane fluid (FS 1265) than when it was suspended in water. Although an adequate explanation for this increased flux has not yet been developed, this way of obtaining increased flux could be important to designers of controlled-release devices because the choice of suspending fluids could be an additional means of controlling delivery.

Blending polymers of PDMS and PSSS prior to room-temperature vulcanization results in membranes with diffusional characteristics different from either PDMS or PSSS.

Significance to Biomedical Research and Program of the Institute: The study has provided much needed information on the type of polymer to be used with a given steroid to either inhibit or enhance the rate of drug release and is therefore directly relevant to the objectives of the CDB.

Proposed Course: Termination. The major contract objectives have been fulfilled.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Systems Approach to Vaginal Delivery of Contraceptive
Drugs - Methodology and Mechanism for Absorption
Contractor: University of Michigan
Money Allocated: \$126,361 (FY 74 for 2 years)

Objectives: The proposed studies are aimed at developing suitable methodology in an appropriate animal system and obtaining firm, baseline data on vaginal absorption of model compounds and contraceptive steroids. The data generated will be used in delineating the general barrier properties of the vaginal mucosa and in developing quantitative, integrated models describing both the release of drugs from vaginal devices and subsequent drug absorption.

Major Findings: A system was constructed based upon perfusing the drug solution in the vaginal tract. For this purpose, a rib-cage type cell was constructed and surgically implanted in the rabbit prior to an experiment. The experimental method including the surgical operation and the perfusion system was evaluated by absorption experiments using n-butanol-1-¹⁴C as the model solute at 37° C and a perfusion rate of 35 ml. per min. Experiments conducted with a number of rabbits indicated that the day-to-day and animal-to-animal variations were small and that the reproducibility was good. The drug disappearance in the reservoir followed first order kinetics from which the apparent permeability coefficient was calculated. The results indicated that a set of experiments may be carried out on a single animal, and the method generally affords rather high precision.

Taking into consideration all the preliminary results of the vaginal absorption of the n-alkanols and n-butyric acid and their comparison to the rat intestinal and human buccal absorption of these reference compounds, it is postulated that the aqueous pore route is much more significant in the vagina.

Significance to Biomedical Research and Program of the Institute: The study of drug absorption from the vagina can further our knowledge on the characteristics of drug absorption from this local site. The work is directly related to the published purpose of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of an Intra-Uterine Sterilization System for the Female
Contractor: IIT Research Institute
Medical Engineering Center
Money Allocated: \$247,977 (FY 71), \$519,465 (FY 72) 15 months
\$483,769 (FY 74)

Objectives: The purpose of this project is to develop a tubal occlusive device and a hysteroscopic guidance system for its placement in the intramural portion of the oviduct under direct visualization. Since this technique would employ a transcervical approach, no surgery would be required. Instrument and device development will follow a carefully sequenced schedule of evaluation in animal models, extirpated human uteri and finally clinical trials.

Major Findings: The contractor has continued to improve the design of a steerable hysteroscope. The latest model incorporates several new features including enhanced fiberoptic system, extended steerability, improved valve system for flushing and expanding the uterus, device channel capable of handling electrocautery instruments and finally complete sterilizability. The ability to sterilize the hysteroscope by conventional immersion techniques is considered a major achievement for instruments of this general design. Each improvement in instrument design was carefully evaluated in baboons (under actual operating room conditions) and in extirpated human uteri. The contractor is presently developing a dispersive image enhancement technique which will double the resolution and improve image quality significantly.

The latest prototype instrument is now being evaluated in patient volunteers about to undergo hysterectomy at Rush-Presbyterian-St. Luke's Medical Center. Preliminary results indicate that a greater intrauterine pressure than that authorized by the NIH Medical Board will be necessary to expand the uterus sufficiently to identify the uterotubal junctions.

A soft device is being evaluated in the baboon for tissue reactivity, retentiveness and occlusive properties. These devices incorporate one of several types of mechanical aids for device retention.

Significance to Biomedical Research and Program of the Institute: The development of a simple outpatient procedure incorporating the transcervical approach to sterilization is highly relevant to the mission of the CDB.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of a simple non-surgical means of sterilization. Clinical protocols have been approved for Phase I (evaluation of the instrument) and Phase II (insertion of tubal occlusive devices) studies in

hospitalized patients at the time of medically indicated hysterectomy.
Phase I studies are now in progress.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Device and Technique for Blocking Fallopian Tubes
Contractor: Franklin Institute Research Laboratories
Colloids and Polymers Laboratory
Money Allocated: \$78,866 (FY 71), \$124,620 (FY 72) 15 mos.
\$27,694 (FY 74) 6 mos.

Objectives: The purpose of this project is to develop a new device and application technique for blocking the fallopian tubes with medical grade silicon rubber cured in situ. The device and technique are to be evaluated in rabbits for ease of installation, tissue compatibility, efficacy and reversibility and in rabbits and in monkeys for safety.

Major Findings: During the past year the contractors redesigned and fabricated a new detachable obturator tip, improved the non-air-entraining mixer-dispenser and assembled a complete prototype system for polymer installation in human subjects. A ball-tipped fixed curve device was used successfully in carrying out clinically indicated salpingograms (not under this contract) in two subjects. The ball tip seated readily at the uterotubal junction and no backflow into the uterine cavity was observed. The contractor has indicated that the development of several prototype devices for use in the human female has been completed.

A radiopaque system was developed using finely divided silver which permits the monitoring of the occlusive devices in situ by radiography. This could assist in following the extrusion of the elastomer into the oviducts under actual operating room conditions.

The occlusive properties of the cured-in-place silastic were demonstrated in rabbits 34 days following instillation. Neither sperm nor blastocysts were found above the plug 4 days after double mating although two unfertilized ova were seen in one animal. Furthermore, dye injected into the uterus of device bearing rabbits could be forced past the tubal plugs only when great pressures were applied.

Chronic rabbit studies (105-234 days) for long-term retention, efficacy and tissue reaction have been completed. A single pregnancy was observed out of nine rabbits which had two to ten matings each during the study. Implantation was confined to the right uterine horn; no plug was present in the right oviduct.

Oviducts were examined by both SEM and normal light microscopy procedures immediately after device removal and, in some cases, about one month after removal of the plug. No adverse findings were recorded. The loss of isthmial cilia, occasionally seen immediately after device removal, is apparently reversible inasmuch as normal ciliation was found after a one-month "recovery" period.

Chronic (111-182 days) primate studies included 11 rhesus monkeys, four of which had both oviducts occluded, six with unilateral occlusion and one of which had neither oviduct occluded.

The anatomy of the intramural portion of the monkey oviduct necessitated polymer instillation via the abdominal ostium. The 50% expulsion rate included several intraperitoneal expulsions. However, the expulsion rate was probably due to the problems associated with introduction of the polymer from the ampullary (ovarian) end of the oviduct. Composite structures consisting of a human-size obturator tip and a cured silastic thread formed and bonded to the tip were placed in the uterine cavity of three animals. Only one monkey retained this composite device, but it was found in perfect condition 111 days after insertion.

No evidence of cellular pathology or tissue damage associated with the presence of the silastic plug could be demonstrated by SEM or routine light microscopic procedures.

In vitro studies have been undertaken to determine the potential exposure to the stannous octoate catalyst used in curing the elastomer. There was little difference in the concentration of tin (determined by atomic absorption spectrophotometry) between control media (saline) and that exposed to precured or cured in situ elastomer for a period of 24 hours.

Significance to Biomedical Research and Program of the Institute: The development of a simple, inexpensive, nonsurgical means of occluding the oviducts for sterilization of the female is directly relevant to the goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program. Approval for the first clinical trials of the cornuosalpinguide are pending NIH Medical Board review.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of a Reversible Vas-Occluding
Prosthesis Utilizing a Microporous Tissue Interface
Contractor: Tecna Corporation
Money Allocated: \$104,325 (FY 72), \$99,152 (FY 74)

Objectives: The major goal is to investigate the feasibility of utilizing a microporous tissue interface to achieve a reversible vas-occluding prosthesis. To this end MTI-coated tubular prostheses have been fabricated and implanted in animals to develop methods of achieving rapid tissue ingrowth and tenacious adhesion to the vas capable for forming a spermatic fluid seal.

Major Findings: (1) The MTI-coated prosthesis is capable of achieving tissue ingrowth sufficient to fix the prosthesis in place for periods of at least one month and to require appreciable force approximating tissue shear stresses for prosthesis removal. Physical testing and gross observations are supported by histologic data from light and electron microscopy. (2) Two surgical techniques have been developed, at least one of which, transmural insertion, appears to be consistently effective in achieving fixation of the MTI-coated prosthesis. (3) It appears that functionally adequate fixation can be achieved without pretreatment of the inner lumen of the vas to remove mucosa. (4) Patent vas and prosthesis lumens can be maintained (for at least one to two months) if no or mild mucosal treatments are used. (5) No cellular atypia or reactions to the implant have been observed. Reactions to spermatic infiltrates have been seen, but such infiltrates are infrequent and appear to be associated with blocked sperm flow or a loose fit of the prosthesis in the vas.

Significance to Biomedical Research and Program of the Institute: It is important to determine the utility of the MTI-coated prosthesis as the development of devices for male sterilization is directly relevant to the purposes of the Contraceptive Development Branch activities.

Proposed Course: This is expected to be a continuing contractual effort leading towards the development of new contraceptives and is an integral part of the contractive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Design and Development of Implantable Contraceptive Devices for Use in the Male.
Contractor: IIT Research Institute
Money Allocated: \$150,000 (FY 71), \$194,476 (FY 72), \$200,694 (FY 74)

Objectives: The purpose of this project is to develop successful reversible vas occlusive devices, which will be adaptable to long term implantation in the general population. The research proposed includes intensive study of the mechanisms which affect sperm transport and to identify possible remedial changes to improve overall device performance.

Major Findings:

- 1) A total of 31 devices have been implanted in the transected vas mode. Eight of the animals producing viable sperm have been under observation for periods of between six months and one year. Total sperm count and sperm viability, however, are decreased. The biocompatibility of the materials used in the device itself may in some way be modifying total sperm output. This may be of less importance in the human, because physiological differences in the dog appear to include the inability to accumulate and store sperm as in humans.
- 2) Six vasovasotomies were done on dogs with induced periods of aspermia of up to 12 months. The prosthesis devices were silastic rubber with flexible pilot tubes, varying in total length from 1.55 to 4.3 centimeters. Data indicate that sperm transport can be restored in dogs which have been bilaterally vasectomized for periods of up to one year and that devices with sperm path lengths of up to 4.3 cm do not necessarily affect sperm transport in the dog.
- 3) Tissue ingrowth has been carefully evaluated in all transected vas implant devices removed for study. Sperm granuloma, sperm leakage, or perforation damage was not noted in any of the samples.
- 4) Failures have been experienced with the transected vas implants; however, the causes of these failures have been variable and tend to reflect a complication of the surgical technique.
- 5) Restoration of sperm transport occurred in two animals in which the device was implanted in the closed position for several weeks and then opened. The evaluation of these device cycling experiments is continuing.
- 6) A new series of implants using the longitudinal technique has been started and is currently under evaluation. The devices retain the soft flexible pilot tubes and the tissue ingrowth materials to enhance

the Ulsane attachment. Transport of motile sperm through these devices have been achieved.

Significance to Biomedical Research and Program of the Institute: The design and development of implantable devices of male sterilization is directly relevant to the purposes of the Contraceptive Development Branch activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contractive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of New Sterilization Techniques
Contractor: Abcor, Inc.
Money Allocated: \$345,000 (FY 71) for two years; \$190,000 (FY 74)

Objectives: The purpose of this project is to develop (1) a reversible intravasal plug with a hollow center into which a removable pin can be inserted, and (2) an absorbable intravasal stent that would facilitate reanastomosis of the cut end of the vas following vasectomy. The effectiveness of these new sterilization techniques will be studied in the guinea pig and monkey.

Major Findings: Absorbable Intravasal Stent: Progress to date on the development includes the use of starch, collagen and a copolymer of lactic-glycolic acid. A total of 87 guinea pig studies have been performed with each of the materials. Preliminary implantation studies indicate absorption occurs using all materials. Regeneration of the vas took place along the stent up to a distance of 4 mm. Gross and histological examination showed little or no adverse effects. Breeding studies with males implanted with starch and polyester stents resulted in 12/13 pregnancies with starch and 4/5 pregnancies with polyester.

Reversible Intravasal Plug: An intravasal plug with a hollow center from which the pin can be removed has been developed. The outer wall of the plug has a fine layer of flock for tissue ingrowth. The normal insertion of the plug is with the removable pin in place; the pin can be removed later. The plug is inserted in the lumen of the vas through an opening in the vas wall. A number of inflammatory agents were used on the flock material to enhance epithelial destruction for tissue ingrowth. Quinacrine appears to offer very promising results.

Significance to Biomedical Research and Program of the Institute: It is important to determine the utility of these contraceptive devices as the development of devices for male sterilization is directly relevant to the purposes of the Contraceptive Development Branch activities.

Proposed Course: This is expected to be a continuing contractual effort leading towards the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Use of Reversible Vasectomy Device in the Guinea Pig
Contractor: New York Medical College
Money Allocated: \$70,000 (FY 71), \$59,933 (FY 72), \$68,623 (FY 74)

Objectives: The purpose of this project is to ascertain the usefulness of a reversible intravasal valve as a contraceptive device in the male guinea pig.

Major Findings: (1) Two types of Bionyx Control devices have been implanted into a total of 26 guinea pigs in the "off" position. The animals were aspermic by the 9th week and some have remained so for periods up to 60 weeks. Thirteen of these aspermic animals were turned "on" and put through sperm in their ejaculates. (2) A total of 23 guinea pigs have been implanted with the devices in the "on" position. Twenty of these animals put through sperm in their ejaculates. Three "on" animals did not put through sperm and upon surgical exploration, it was found that the devices were improperly implanted. Once corrected, sperm appeared. Sperm have been observed for periods of up to 60 weeks. Ten of these animals were turned "off" and became aspermic." (3) No evidence of tissue necrosis, sperm granuloma formation, or "device rejection" has been noted during the tests. There was no corrosion of the devices noted during the in vivo tests over a 27-month period, nor in in vitro tests carried out in serum and in Ringers' solution. (4) Five animals that put through motile sperm with the device in the "on" position were placed in mating tests. Seven births (20 offspring) have resulted so far.

Significance to Biomedical Research and Program of the Institute: It would be important to determine the usefulness of this contraceptive device, as the development of devices for male sterilization is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading towards the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT

July 1, 1973 through June 30, 1974

Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Clinical Studies of Estrogens as Postcoital Contraceptive Agents
Contractor: Planned Parenthood of Minnesota
Money Allocated: \$52,662 (FY 73) 2 Yrs.

Objectives: The purpose of this project is to study the safety and efficacy of ethynyl estradiol as a postcoital contraceptive. A standard dose and uniform protocols are employed in patients requesting this form of contraception who meet the rigid criteria for acceptance into the program. The study is part of a larger program designed to acquire sufficient data on ethynyl estradiol to support or refute its use as a postcoital contraceptive. The investigation is being carried out under IND #9525.

Major Findings: A single pregnancy has been observed in the first 100 cases following the oral administration of 5 mg. ethynyl estradiol daily for 5 consecutive days starting within 72 hours of unprotected midcycle coital exposure. While this would appear to be a "method failure" the possibility of "patient failure" can not be ruled out at this time. The major side effects reported were nausea, vomiting and abnormal menses. No serious untoward reactions have been encountered.

Significance to Biomedical Research and Program of the Institute: The development of new and more acceptable means of postcoital contraception is directly related to the goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort until sufficient data is generated by the total program to support or refute the use of ethynyl estradiol as a safe and effective postcoital contraceptive agent.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Clinical Studies of Estrogens as Postcoital Contraceptive Agents
Contractor: Kapiolani Hospital
Money Allocated: \$10,400 (FY 73) 2 Yrs.

Objectives: The purpose of this project is to study the safety and efficacy of ethynyl estradiol as a postcoital contraceptive. A standard dose and uniform protocols are employed in patients requesting this form of contraception who meet the rigid criteria for acceptance into the program. The study is part of a larger program designed to acquire sufficient data on ethynyl estradiol to support or refute its use as a postcoital contraceptive. The investigation is being undertaken under IND #9658.

Major Findings: No pregnancies have been observed to date following the oral administration of 5 mg. ethynyl estradiol daily for 5 consecutive days starting within 72 hours of unprotected midcycle coital exposure. The major side effect of this treatment is nausea. No serious untoward reactions have been encountered.

Significance to Biomedical Research and Program of the Institute: The development of new and more acceptable means of postcoital contraception is directly related to the goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort until sufficient data is generated by the total program to support or refute the use of ethynyl estradiol as a safe and effective postcoital contraceptive agent.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Clinical Investigation of Ethynyl Estradiol and Conjugated Natural Estrogens as Postcoital Contraceptive Agents
Contractor: Planned Parenthood League of Connecticut, Inc.
Money Allocated: \$132,342 (FY 74) 2 years

Objectives: The purpose of this project is to study the safety and efficacy of ethynyl estradiol and conjugated natural estrogens as postcoital contraceptives. Standard doses of both drugs and uniform protocols will be employed in patients requesting this form of contraception who meet the rigid criteria for acceptance into the program. The study is part of a larger program designed to acquire sufficient data on ethynyl estradiol and conjugated natural estrogens to support or refute their use as postcoital contraceptive agents. The investigation will be carried out under IND #10,138.

Major Findings: This contract was awarded only recently and no clinical studies have been completed.

Significance to Biomedical Research and Program of the Institute: The development of new and more acceptable means of postcoital contraception is directly related to the goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort until sufficient data is generated by the total program to support or refute the use of ethynyl estradiol and conjugated natural estrogens as safe and effective postcoital contraceptive agents.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Study of the Safety and Efficacy of a Conjugated Estrogen Preparation as a Postcoital Contraceptive
Contractor: University of Florida
Money Allocated: \$53,086 (FY 73) 2 Yrs.

Objectives: The purpose of this project is to study the safety and efficacy of a conjugated estrogen preparation as a postcoital contraceptive. A standard dose and uniform protocols are employed in patients seeking this form of contraception who meet the rigid criteria for acceptance into the program. The study is part of a larger program designed to acquire sufficient data on conjugated natural estrogens to support or refute their use as a postcoital contraceptive. The investigation is being undertaken under IND #9839.

Major Findings: No pregnancies have been observed to date following the oral administration of 30 mg. conjugated natural estrogens daily for 5 consecutive days starting within 72 hours of unprotected midcycle coital exposure. The major side effect of this treatment is nausea. No serious untoward problems have been encountered.

Significance to Biomedical Research and Program of the Institute: The development of new and more acceptable means of postcoital contraception is directly related to the goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort until sufficient data is acquired by the total program to support or refute the use of natural conjugated estrogens as a safe and effective postcoital contraceptive agent.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Clinical Evaluation of a Fluid-Filled Intrauterine Device
Contractor: Tecna Corporation and University of California
Money Allocated: \$51,980 (FY 74)

Objectives: The major objective of this contract is to clinically evaluate a new fluid-filled intrauterine device (IUD). Preliminary data indicate that the device is well tolerated and provides high contraceptive efficacy. The IUD will be inserted into 100 women for an anticipated 1050 women months. This represents a minimal sample upon which statistically valid conclusions can be made.

Major Findings: The IUD has been inserted into 77 subjects, 69% of whom were nulliparous. Total experience with the device under this particular study amounts to 350 woman-months of use. Additional subject experience is available from non-federally supported sources. Preliminary data indicate that in terms of pregnancy rates, expulsions and removals the fluid-filled IUD represents an improvement over the Lippes loop. Considerably more extensive data are required to substantiate the initial findings.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive devices is directly related to the published purpose of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptive devices.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Contraceptive Development Studies for Males: Oral Steroid Hormone Administration
Contractor: University of Washington School of Medicine
Money Allocated: \$118,056 (FY 71) for two years, \$58,315 (FY 74)

Objectives: This contract proposal is designed to evaluate the combined effects of two androgenic steroids on spermatogenesis when administered to normal adult human males. The ultimate goal is to develop an effective oral contraceptive technique for males.

At the present time there is no compound available for effectively inhibiting human spermatogenesis which is non-toxic or absent from adverse reactions which would render the preparation either unacceptable to the patient or to his physician.

Major Findings: The first extensive male contraceptive trial is nearing completion. It involves the use of Danazol in combination with either methyl testosterone (MT) or testosterone enanthate (TE). The Danazol and MT are given orally daily and the TE is given as a once-a-month injection. Neither one of the three drugs proved useful when given alone. Likewise, Danazol together with MT at 10mg/day had little effect on sperm production. The most effective treatment involved Danazol-600mg/day together with TE-200mg/month. In this group 10 out of 15 subjects had sperm counts of 5 million or less per ejaculate.

No undesirable side effects were observed nor were there any toxicological manifestations among the volunteers. It is also important to know that male volunteers are readily available for participation in projects dealing with the development of male contraceptives.

Significance to Biomedical Research and Program of the Institute: Development of contraceptive techniques for the male is highly relevant to the contraceptive development program.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Distribution of Hormonal Substances
Contractor: Emory University
Money Allocated: \$11,338 (FY 73)

Objectives: The objective of this contract is to provide facilities to store, package and distribute hormonal substances procured under CDB contracts to qualified investigators requesting the materials for research purposes.

Significance to Biomedical Research and Program of the Institute: The hormonal substances being distributed play an indispensable role in the reproductive processes. Their distribution and subsequent utilization furthers our understanding of reproduction and may lead to the development of new contraceptives. The latter is a stated goal of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974

Population and Reproduction Grants Branch
Center for Population Research

The Population and Reproduction Grants Branch within the Center for Population Research provides support for fundamental biological and chemical research and training in biomedical disciplines dealing with reproduction and behavioral-social disciplines dealing with the determinants and consequences of population phenomena.

The appropriation for FY 74 includes three categories of funds: 1) the President's Budget of \$17,663,000 for regular research programs and \$682,000 for special research (RCA's and RCDA's); 2) the Congressional Increase of \$5,154,000 for regular research and \$77,000 for special research programs; and 3) the training program of \$1,843,000 of which \$1,117,000 is for training grants, \$530,000 for F22 new-type fellowships, and \$196,00 for F2 and F3 old-type postdoctoral and special fellowships. These do not include the so-called "impounded funds" disbursed in 1974 on FY 73 fundable unpaid grant applications of \$4,899,900. The Congressional Increase was used to fund projects for a 24-month period, instead of 12 months, by administrative decision.

As of February 1974, grant funding of \$23,576,000 (President's Budget plus the Congressional Increase) supported 308 Project Research Grants (\$17,393,134); 10 Centers (\$2,702,967), 10 Program Projects (\$2,720,899), and 25 Research Career Programs (\$759,000). One program project (Biggers, Harvard) was supported with FY 73 impounded funds. Training funds of \$1,843,000 supported 14 Training grants (\$1,117,000), 40 F22 New Fellowships, \$530,000. Projects were supported in areas of reproductive biology and contraceptive development, animal ecology and behavior and in the behavioral-social aspects of population research. While no new training awards had been made after January 29, 1973 the current fiscal year has seen the birth of a new fellowship program (F22) as well as the payment of fundable training grant programs from FY 73 impounded funds.

The mission of the Branch was broadened in July 1970 with the development of the Population Research Center Award Program, currently funding ten such Centers at a level of \$2,702,967, which represents 9 percent of the total Branch budget and 10 percent of the research budget. The program provides additional core but not research, support together with new program developments for investigators conducting interdisciplinary funded research relevant to population problems. Population Research Centers are designed to facilitate multidisciplinary communication and effort in the population field, give visibility to research areas, and provide administrative support and a physical environment conducive to scientific productivity. Centers have been established at: The Population Council, New York City, Biomedical Division; University of Texas, Population Research Center; Vanderbilt University, Department of Obstetrics and Gynecology; University of Wisconsin, Center for Demography and Ecology; Harvard University Medical School, Center for Population Research; University of Chicago, Ben May Laboratory; Johns Hopkins University, Department of Population Dynamics; Baylor College of Medicine, Department of Cell Biology; University of North

Carolina, Carolina Population Center; and Columbia University, Department of Obstetrics and Gynecology. The Centers at the Universities of Texas, Wisconsin and North Carolina are in the behavioral-social field, the one at Johns Hopkins University involves both fields, while the others relate to the biomedic field.

In contrast, Program Project Grants provide support for component research projects, as well as core facilities. This multidisciplinary type of research effort involves the concerted attack of scientists in different, but related, disciplines on a common well-defined goal or problem area. Program Projects are located at: Columbia University, Department of Obstetrics and Gynecology; University of Michigan, Department of Pathology; Northwestern University, Department of Physiology; Oregon Regional Primate Research Center, Department of Reproduction; University of Pennsylvania, Department of Obstetrics and Gynecology; University of Texas at Austin, Department of Zoology; University of Texas Medical School at Houston, Program in Reproductive Biology and Reproductive Endocrinology; Case Western Reserve University, Department of Reproductive Biology; University of California at San Diego, Department of Biology; Washington University School of Medicine at St. Louis, Department of Obstetrics and Gynecology; and Harvard University Medical School, Laboratory of Human Reproduction and Reproductive Biology. These programs contain 64 individual projects, each with an average support of \$42,514. Since the establishment in 1970 of the Research and Training Committee, the program projects and centers programs (P01) have grown from \$0.9 million to \$5.6 million. At present, this represents about 21% of the Branch's research budget; approximately equal amounts are being spent on each type of grant. Total funds for P01 programs have increased from 10.3% of total research funds in FY 70 to 20.9% in FY 74. The growth in this period took place in the centers program which increased from about 2.5% to 10% of the research budget, while the funds for program projects have remained relatively constant at approximately the 10-11% level. If the P01 applications are broken down into their component projects, the overall approval rate (53%) of these projects is slightly lower than that of regular research grant applications (60%).

An annual meeting of the Directors of the Population Research Centers and Program Projects is held in July of each year at one of the Centers to facilitate the exchange of information regarding new and significant developments in reproduction and behavioral-social aspects of population research. An annual progress report is published as well as papers presented at a conference held in conjunction with the annual meeting. The first scientific conference, held at Vanderbilt University, July 1972, was organized by Dr. B. O'Malley and featured "Receptors for Reproductive Hormones". It dealt with the role of receptors in the mechanism of action of both sex steroid and gonadotropic hormones. The papers presented at the Conference were published by the Plenum Press, New York City. At the time, Dr. B. O'Malley was director of the Center at Vanderbilt University, but has since moved to the Baylor College of Medicine, Houston, Texas.

Dr. W. Montagna, former Director of the Program Project in Primate Reproduction at the Oregon Regional Primate Research Center, Beaverton, Oregon, hosted the Second Annual Director's Conference in July 1973. With the title of "Reproductive Behavior", this conference dealt with the neurohormonal and pharmacological determinants of behavior, hormonal development of sexual behavior and

the social behavioral aspects of human fertility. A volume of contributed papers will soon be published by the Plenum Press, New York City. A third conference to be held at the Annual Meeting of the Directors in July 1974, will feature the topic of "Fertility Regulation Through Basic Research", and will be held at the Population Council, The Rockefeller University, New York City. Host for the meeting will be Dr. S. J. Segal, Director, Biomedical Division. The program includes papers on gonadotropin release and receptor site interactions, implications for fertility regulation, recent advances in fertility regulation, fertility regulation by suppression of gamete development and function, and the molecular biology of fertility regulation.

The annual reports of accomplishments in population research at the population research centers and program projects consist of summaries of the objectives of each program, the disciplines and staffs represented, the core facilities being supported, and brief descriptions of the activities and research achievements in reproductive biology and social science research.

Impounded FY 73 funds made available in FY 74 amounted to \$4,899,000. Of this, \$2,993,758 supported 63 research grants (as of May 1) approved in FY 73 (some applications are still under administrative review). Of the 63 funded applications, 21 deal with behavioral-social topics and 42 with biomedic ones. This is about the same division of the subject areas which exists in the regular FY 73 and FY 74 categories. \$206,242 funded one program project (Harvard), and \$132,000 supported research career programs. Training grant support amounted to \$1,250,000 and that of F2- and F3-fellowships totaled \$317,000.

The Research Career Program (RCP) includes two types of awards (FY 74 funding, \$759,000): the Research Career Development Awards (RCDA), \$715,000, and the Research Career Awards (RCA), \$64,000. Two scientists are recipients of the latter: Dr. M.C. Chang, Worcester Foundation for Experimental Biology; and Dr. Arpad I. Csapo, Washington University School of Medicine. An additional \$132,000 became available in FY 74 from impounded FY 73 funds for RCDA awards approved at fundable level in FY 73. In April, 1974, a continuation of the NIH Research Career Development Program was announced; this program was being phased out at the time. Awards in this program are to foster the development of promising young scientists with outstanding research potential for careers of independent research in the sciences related to health. The Population and Reproduction Grants Branch, CPR, is the only program within the NICHD to express interest in continuing this type of training.

Fellowship Programs. During the year a new type fellowship was introduced for the first time. This new fellowship commonly referred to as type F22 has several new features, the most prominent being a base stipend of \$10,000 with certain pay back features if research is not the end purpose of the training. The Branch funds 40 of these F22 fellowships, which vary in amount, for a total of \$530,000. In addition 9 of the old type fellowships, F2 and F3 postdoctoral and special, are supported for a total of \$196,000, the average stipend being \$21,777. The funds spent for all types of training during FY 74 amounted to \$2,602,000.

Training Programs. The six training programs currently receive support of \$1,117,000: University of Pennsylvania, Reproductive Physiology; Princeton

University, Special Training in Demography; University of Chicago, Social Science Aspects of Fertility and Family Planning; University of Washington, Endocrinology of Reproduction; Case Western Reserve University, Biology of Human Reproduction; and University of Massachusetts, Mammalian Reproduction.

After commitment of these "old" training programs had been renewed, the Branch was able to fund the following three new training programs: Baylor University, Reproductive Biology: Regulatory Mechanisms; Washington University, Training in Reproductive Biology; University of Maryland, Training Program in Reproductive Endocrinology. In addition the Branch was able to fund an additional rather large "old" training program at the University of North Carolina, in Population Studies.

Staff. During this year, Ms. Julia Lobotsky, M.S., joined the staff in August as Biologist. She came to the Institute from the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, where she held a position as a research scientist with speciality in reproductive biology and was also editor of a scientific newsletter dealing with research in prostaglandins. Two scientists, enrolled in the Grants Associate Program, NIH, spent 3 to 4 weeks in the Branch: Elke Jordan, Ph.D., and Andrew Chiarodo, Ph.D. Dr. Chiarodo's report on the "Funding and Review Status of PRGB Population Research Centers and Program Projects" forms a part of this report. Dr. Allyn J. Waterman's appointment as a Consultant to the Center following his retirement in August, 1972, was terminated in September with his temporary appointment as Health Scientist Administrator. Dr. W.H. Spillane, Medical Social Worker, resigned and is currently Chief, Programmatic Information Section, Drug Abuse Information Branch, Alcohol, Drug Abuse and Mental Health Administration.

Symposia and Workshops. A symposium was held on the "Mechanism of Ovulation" in conjunction with the sixth annual meeting of the Society for the Study of Reproduction at the University of Georgia, Athens, Georgia. Organized and directed by Dr. A.V. Nalbandov, University of Illinois, President of the Society, it encompassed such topics as: role of hormones in the maturation of oocytes, binding of gonadotropic hormones by follicle cells, role of steroid synthesis in the process of ovulation, ovarian proteolytic activity and ovulation, effect of LH in the follicle wall, effect of FSH and LH and their antibodies in follicle growth and ovulation, and the role of nerves and catecholamines in ovulation. The proceedings appeared in the March 1974 issue of the Biology of Reproduction, the Society's journal. This symposium continues a series supported by the Population and Reproduction Grants Branch. Previous topics in the series have included: male reproduction, gametes and fertilization, environment and reproduction, immunoreproduction and the corpus luteum.

A conference on "Biorhythms and Human Reproduction" sponsored by the International Institute for the Study of Human Reproduction, Columbia University, and the Center for Population Research, NICHD was held at the Sterling Forest Conference Center. Organized and directed by R.L. Vande Wiele, M.D. and R.M. Richart, M.D., and with more than 70 scientists from the United States and abroad participating, this conference examined carefully selected subjects in human reproduction, emphasizing those that in recent years have undergone rapid growth and development. The publication, edited by Michel Ferin, M.D., Frank Halberg, M.D., Ralph Richart, M.D., and Raymond Vande Wiele, M.D., covers such

topics as: mathematical models and special structures, primary and secondary rhythms related to the menstrual cycle, the hypothalamic-pituitary-gonadal and adrenal axes, testicular function, synchronizers of reproductive function, characteristics of biorhythms, cellular and neuroendocrine mechanisms involved in biorhythms.

Sponsored by the Reproductive Biology Study Section, NIH, and the Center for Population Research, a workshop on the "Uterus" was held in conjunction with the April 1974 meeting of the Study Section. Co-chair persons were Stanley G. Korenman and Helen A. Padykula. Topics discussed included: uterine structure, uterus as a model of hormone action, uterus as a muscle, uterine fluid proteins, and clinical status of prostaglandins. No publication is anticipated.

In June 1974 the Society for Developmental Biology and the Center for Population Research co-sponsored a "Symposium on Reproductive Biology" at the time of the 33rd annual meeting of the Society at the University of Georgia. A publication will be forthcoming; topics discussed included: gametogenesis, early mammalian development, sex differentiation and behavior, implantation, and social and biological controls of reproduction.

In order to meet the continually growing needs for research and teaching in the behavioral-social aspects of population research, the Center for Population Research sponsored a Summer Institute in Population held at the Carolina Population Center, University of North Carolina for four weeks, beginning July 30, 1973. The Institute was developed to help meet needs for increased population research and teaching in five areas: anthropology, economics, political science, psychology, and sociology.

Highlights of PRGB Supported Biomedical Research

Beyond the need for goal-oriented research associated with population problems arising with the significant expansion of the world population, there is an undisputed need for continued support of fundamental investigations which will furnish basic information concerning reproduction and population dynamics. Studies at the biochemical, genetic, physical, physiological, behavioral, and early developmental levels in both the normal and the pathologic, in vivo and in vitro, the vertebrate and the invertebrate are continuing to help towards an understanding and appreciation of human reproduction and sexual development. Support for around 400 research grants has enabled basic biomedical and behavioral scientists to identify their research interests in population research and basic reproductive biology with the mission of the Center. The discovery of new, safe, more effective and acceptable methods of regulating family size will require the cooperative efforts of biomedical and social scientists from many different disciplines including: biology, chemistry, physics, bioengineering, sociology and psychology. The problems associated with human infertility continue to be of concern; failure to have children can be as disturbing to healthy family life as too many or too closely spaced children. The field of male fertility has been quite neglected, for various reasons, including the difficulty involved in research in this problem area, as well as the relatively fewer points in the male reproductive system available for interruption.

A survey by title of the currently supported research grants in the biomedic field shows investigations being conducted within the broad program guidelines from germ cell development to implantation in placental mammals, including the human. Since the last annual report there have been numerous developments in the basic reproduction field among our grantees. Areas showing particular progress and promise basic to contraceptive development include: gamete development and maturation, gene activation and other aspects of the mechanism of action of hormones at subcellular levels, hormone receptors, ovulation, gamete transport, fertilization, implantation, corpus luteum initiation and maintenance, biorhythms, oviduct, uterine proteins, spermatogenesis, vasectomy, neuroendocrinology, hypothalamic-hypophyseal-gonadal circuit, pineal gland, and pheromones.

Ovary. Cells from large follicles of the pig bind many times more labelled hCG than do cells from small follicles. Whether these sites are on the same or different cells or cell types is not known. LH increases cyclic AMP levels in cultured granulosa cells from large follicles but not from small follicles (Channing and Kammerman). The inability of LH to stimulate cAMP or progesterin secretion by the granulosa cells of small follicles may be due to a deficiency of LH receptors at this stage. There appears to be an inhibitory action of follicular fluid upon luteinization. Granulosa cells from large follicles of the pig can luteinize spontaneously in culture; cells from small follicles do not. Follicular fluid from small follicles appears to act at a step after LH binding (Ledwitz-Rigby, Channing). Granulosa cell maturation may be a process which involves loss of available FSH receptors. The maximum capacity for FSH binding was observed in small follicle granulosa cells. Conversely, the binding capacity for hCG was greatest for large follicle granulosa cells (Midgley, Desjardins, et al).

Blandau and colleagues have found an acid proteinase, perhaps similar to cathepsin-D, in the preovulatory follicular stigma area (rabbit). They have also confirmed the presence of mammalian collagenase in the walls of the stigma of the preovulatory follicles, which is involved in the dissolution of connective tissues in the ovulatory process, depolymerizing the thick layer of collagen in this region.

Prostaglandin E₂ stimulates steroidogenesis in vitro in human corpus luteum slices to an extent similar to human chorionic gonadotropin (hCG) (Marsh, LeMaire). This prostaglandin, as well as hCG and hLH, also increase the incorporation of labelled adenine into labelled cyclic AMP by stimulation of adenyl cyclase rather than inhibition of the destructive phosphodiesterase. Corpora lutea of the menstrual cycle are much more responsive to stimulation in terms of steroid synthesis and cyclic AMP formation than corpora lutea of pregnancy.

Oviduct. A pro-ovarian ciliary current has been found in pig and rabbit oviductal isthmus that provokes rotational turbulence favoring fertilization and the deposition of egg coatings (Blandau et al). Egg transport over the fimbrial surface is affected by the number of ciliated cells (hormone dependent). Ampullary muscle contractions were more coordinated and vigorous in rabbits at the time of gonadotropin-induced ovulation or after progesterin injection. This and other evidence indicated a relation between transport and an increase in the secretion of progesterin and a fall in the level of estrogen in the estrogen-primed smooth musculature of the ampulla. The new technique of the scanning electron microscope demonstrates most convincingly the changes in the oviduct

due to estrogen. The fimbriae from oviducts of spayed rabbits showed extensive loss of cilia and flattening of the secretory cells; administration of estrogen caused almost complete reciliation (Rumery, Eddy).

Uterus. Progesterone-binding components have been found in the uterine cytosol from mouse and rat (O'Malley et al). The amount of binding is dependent upon the animals' endocrine status. Furthermore, the progesterone binding capacity can be transferred from target tissue chromatin. When these acceptor sites are transferred, there is also a corresponding transfer of the receptor-binding capacity without alteration indicating that the acidic protein fraction defines the specificity of the acceptor site for the steroid hormone-receptor complex. Perhaps the target cell genome contains specific acceptor sites which receive the inducer complex as it enters the nucleus.

Induction of blastokinin, protein specific to the preimplantation uterus, has been demonstrated in rabbits treated with oral contraceptive steroids containing the C-19 methyl group, but not with those which are 19- nor steroids (Arthur and Chang). Asynchrony between embryonic development and an altered uterine environment has been suggested as a mode of action for steroidal contraceptives.

Blastocyst and Implantation. The rabbit blastocyst contains enzymes capable of synthesizing cholesterol and pregnenolone from acetate and metabolizing various steroid hormones (Huff and Eik-Nes). Significant concentrations of various progestins have been found in the blastocyst and its fluid, possibly provided by endometrial secretion. Unconjugated estrogen and progesterone have been found in pig blastocysts, and it seems possible that steroid hormones are synthesized by the blastocyst (Perry, Heap and Amoroso). Whether these locally produced hormones play a role in implantation is not known.

The use of antibodies directed against hormones or tissues essential for reproductive functions has been studied for a long time and is an extremely useful methodological tool. Antisera to rat median eminence terminate gestation and inhibit ovulation in the rat; these effects could be reversed by simultaneous administration of LH or LRH (Ying, Greep). Antiserum produced against rat deciduoma contains antibodies specific to rat decidual tissue antigens. When administered to pregnant rats, it causes abortion. Histologically, the trophoblastic giant cells degenerate first and this causes death of the embryo and later degeneration of decidual tissue and placental separation (Yoshinaga).

Testis. Research on the male reproductive system continues to be active and now we are beginning to learn what the role of FSH is in the male. It has been known that testicular androgen is necessary for the completion of spermatogenesis in the seminiferous tubules and for the sperm in the epididymis to acquire fertilizing capacity. Androgen, therefore, must be carried from its cellular origin in the testis to the sites of action. There appears to be a transportable androgen binding protein (ABP) that is produced by Sertoli cells under the influence of FSH. It provides more binding sites for androgen within the seminiferous tubules and it is carried by rete testis fluid to the epididymis so that a high concentration of androgen is also available there. ABP is not the same protein as cytosol receptor protein. LH as well as FSH is needed for the completion of spermatogenesis; it may be that LH acts indirectly by stimulating the secretion of testosterone (French, et al). There are studies to

identify the cellular site of action of FSH in the testes and the biochemical and morphological events which occur at this time (Means, Huckins). The germinal epithelium of the testis develops in a milieu with a high testosterone concentration. Testis fluid collected from ligated ductuli efferentes in the rat has a concentration of testosterone about 18 times greater than that in peripheral plasma (Harris and Bartke).

The capacity of testicular gonadotropin receptors to bind hCG and rFSH seems to be related to the developmental status of the steroidogenic and spermatogenic elements of the testis. rFSH-binding increased from 5-15 days of age and remained unchanged through 90 days. hCG, however, showed increasing binding during development with a plateau at 55 days (Desjardins, Zelesnik, Midgley, Reichert).

The structural basis of the blood-testis barrier resides in the occluding junctions between myoid cells surrounding the seminiferous tubule, and occluding junctions between adjacent Sertoli cells. Vasectomy in the dog impaired spermatogenesis in many of the testicular tubules. Vasectomy may affect the integrity of the blood-testis barrier in the dog, in contrast to the rat, and in turn the passage of materials between the Sertoli cells (Heidger).

Studies on the effect of various steroids on the maturation of spermatozoa in the epididymis of the hamster show that normal fertilizing ability in castrated hamster is maintained by androgens in this order of efficacy: 3 alpha-androstanediol, 5 alpha-dihydrotestosterone, testosterone. If males are treated with progesterone, testosterone is unable to overcome its adverse effects but the other two androgens can do so (Lubicz-Nawrocki, Chang).

Prolactin. The control of the release of prolactin is not well defined but a number of studies are addressing this subject. Artificial cervical stimulation (or infertile mating) results in a prolonged period of progesterone secretion by the corpora lutea (around 12 days of pseudopregnancy) as compared to the unmated rat with an estrous cycle of 4 days. Ovarian steroids are known to play an important part in prolonging the prolactin surges once they have been initiated by cervical stimulation (CS). There are two daily surges of prolactin secretion, one diurnal and one nocturnal. It has now been shown that prolactin secretion can be initiated by CS and maintained in the absence of steroid hormones (ovariectomized, adrenalectomized rat). In addition, the surges of prolactin seem to be related to time of day rather than to the time that CS is applied (Smith, M.S. Neill).

The role of brain catecholamines and hypophysiotropic hormones in the release of prolactin is being studied in ovariectomized rats. It appears that prolactin release is under a dual dopaminergic control exerted at both the hypothalamus and the pituitary. Dopamine inhibition of prolactin release may be via activation of cyclic AMP and may be modulated by an increase in prostaglandin E₁ (Ojeda, Harms, McCann; Carr, Conway, Voogt). Thyrotropin releasing hormone (TRH) appears to act directly on the anterior pituitary to increase the release of prolactin (Porteus, Malven). Thus, it would seem that prolactin may have a dual control: dopamine being an inhibiting factor and TRH a releasing factor of prolactin.

Gene Regulation. The fundamental problem of the molecular mechanism of gene regulation during oogenesis and early embryonic development continues to be productively pursued. Mature oocytes of many animal species contain information RNA which is not utilized for protein synthesis until after fertilization. Gene transcription is being studied in a unique model, the Echiuroid worm (Urechis) which has no organized ovary and hence mature eggs are readily available (Davis, F.C.). Studies with Xenopus and sea urchins show that there appears to be a common pattern of arrangement of DNA sequences such that repetitive sequences are interspersed with non-repetitive sequences (Davidson).

Sexual Behavior and Animal Ecology. Research in the area of hormonal control of sexual behavior in animals has resulted in the demonstration of significant neuroendocrine relationships. Lesions in the area of the brain concerned with mating behavior in the female rat abolish this behavior whereas implantation of estrogen elicits mating behavior. LH-RH may have a separate effect on mating behavior aside from its action in releasing gonadotropins (Moss and McCann). Ovariectomized, estrogen primed females treated with progesterone and LH-RH gave positive reactions when placed with a sexually active male, but not those treated with FSH and LH. This suggests that LH-RH may exert a facilitatory effect on the induction of mating behavior similar to progesterone and estrogen.

Observational studies correlating social behavior and territory involve a number of animal species (Emlen, Wagner). The social milieu or the density of population can affect the development of normal male reproductive patterns.

Puberty. Estrogen is an important factor in the maturation of the hypothalamic-anterior pituitary complex in development and in regulation of the onset of puberty. In the rat "matured" hypothalamic estradiol receptors appear gradually after birth, then rapidly increase in number between 14 and 21 days of age and reach a maximum at 28 days, indicating the development of the receptors in the hypothalamus at prepubertal stages. Concentration of the estrogen-binding protein in the uterus of prepubertal rats increases rapidly between days 1 and 10 after birth, after which it remains relatively constant (Gorski et al).

Highlights of PRGB Supported Behavioral-Social Research

The behavioral-social population research grants program has had the objectives of stimulating the development of research grant applications, of increasing and diversifying the behavioral disciplines engaged in such research, and of broadening the substantive scope of the program. For the first time since the initiation of the behavioral-social population research grants program in 1968, the program has shown a drop in the number of research grant applications. The number of applications submitted since fiscal year 1970 are as follows: FY 1970 - 25; FY 1971 - 125; FY 1972 - 151; FY 1973 - 228; FY 1974 - 164. After a substantial increase of 77 applications from FY 1972 - FY 1973, there was a smaller decrease of 64 applications from FY 1973 - FY 1974. The decrease may underline the point that most of the behavioral sciences are still in the early stages of their development of population research.

However, the behavioral-social research grants program continues to involve a large number of disciplines in the submission of applications. During the fiscal year, members of 21 behavioral disciplines developed applications, the

same number as in FY 1973. The disciplines were represented as follows (the FY 1972 representation is in parentheses): Economics - 23% (29%); Psychology - 15% (19%); Political Science - 14% (5%); Sociology - 12% (15%); Anthropology - 10% (9%); Demography - 8% (5%); Geography - 4% (3%); Statistics - 2% (3%); Psychiatry - 2%; History - 2% (1%); Epidemiology - 2%; Obstetrics and Gynecology - 1% (1%); and Engineering - 1%. These disciplines had one proposal each: Social Work, Pediatrics, City Planning, Mathematics, Physiology, Genetics, Communications, and Nursing.

Classifying the number of applications in four major substantive areas shows the following: Fertility, 63; Population Growth, Movement, and Composition, 68; Family and Women's Status, 18; Population Policy, 15; total 164.

The pattern of substantive areas shows a change from the usual preponderance of fertility applications to relatively more emphasis on population growth and movement and on population policy, the latter reflecting interest on the part of the political scientists.

Within each of the four major classifications the following sub-categories were represented:

| Fertility | | Population Growth, Movement, and Composition | |
|------------------------------|-----|--|-----|
| Social-cultural factors | 33% | Growth and decline | 44% |
| Psychological factors | 20% | Movement (migration) | 31% |
| Economic factors | 17% | Composition | 19% |
| Abortion | 10% | Density | 6% |
| Sterilization | 8% | | |
| Family Planning | 8% | | |
| General | 3% | | |
| Family and Women's Status | | Population Policy | |
| Family and Fertility | 67% | Population Policy | 10% |
| Women's status and fertility | 33% | | |

The following refers to some of the behavioral-social applications funded during FY 1974, by classification and sub-category.

Fertility

Social-Cultural Factors. Studies include investigation of fertility and assimilation of the foreign born, testing hypotheses concerning the fertility behavior of minority groups in the United States (Gurak), and of childlessness among black Americans, especially in relation to possible effects of upward social mobility (Mommsen). An intensive examination of nuptiality patterns and their possible determinants is planned (Brandes).

Psychological Factors. A large scale study of motivations for having children (value of children) in the United States is being undertaken (Hoffman and Fawcett). An investigator is exploring values and processes underlying

birth planning decisions (Townes), while another is attempting to develop predictive indicators of probable success or failure in contraceptive planning (Oskamp).

Economic Factors. A study of the socioeconomic determinants of post-war fertility in the United States is being initiated (Beaver). An investigator is analyzing the intergenerational transfer of wealth and its possible relations to fertility (Soltow), while another is trying to develop an understanding of the causal relationship between land inheritance and birth rate (Khera). An investigation of the sources of higher black fertility vis-a-vis the white United States population, with special attention to economic and labor force participation factors, is being undertaken (Gregory).

Abortion. A survey of abortion patients in New York City will provide detailed analyses of abortion patients and a large amount of follow-up data on later contraceptive and fertility behavior of women who obtain abortions (Lerner). Another investigator is studying decision-making processes among women with unplanned pregnancies (Rosen).

Family Planning. The analysis of data from an interdisciplinary study of a large scale family planning program in India is being funded (Simmons).

Population Growth, Movement, and Composition

Growth and Decline. Two investigators are attempting to develop better understanding of the dynamics of population growth and decline by studying the causes and effects of population change in the preindustrial setting (Lee), and the relationship between demographic variables and environmental, agricultural, and social organizational processes in a Swiss village over a 300-year period (Netting).

Movement (Migration). An investigator will study patterns of separation of place of work and home in metropolitan areas in the United States (Guest), while another is continuing to study some economic determinants of the choice of residential location in metropolitan areas (Pollakowski).

Density. An investigator is studying the effects of intra-urban population distribution on several social, psychological, and somatic variables and pathologies (Choldin).

Family and Women's Status

Family and Fertility. A study is being initiated of the interrelating variables of kinship, family structure, population and landholding (Sabeen).

Women's Status and Fertility. An investigator is attempting to investigate the effect of such family-building variables as marital status and number of children on the socioeconomic achievement of American female college graduates (Perucci).

Population Policy

Policy. An investigator is concerned with the impact of migration on urban-regional growth, the feedback from growth patterns to migration, and the relation of these factors to Mexican government policy decisions (Greenwood).

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Center for Population Research

The program of the Fertility Regulating Methods Evaluation Branch has followed the lines which were projected in FY 1974 with some exceptions. A new program emphasis was developed to pursue the relationship if any between contraceptive agents and methods and the subsequent occurrence of birth defects. This program consists of several investigations and includes the evaluation of oral contraceptives, the rhythm method, the use of spermicidal agents and the use of IUDs. Both retrospective and prospective investigations are being initiated which will cover those specific malformations which are currently suspected of being associated with specific contraceptive methods like congenital heart disease and malformations of the limb, but also other types of malformations.

The collaborative investigation of the relationship between oral contraceptive steroids and cervical dysplasia was modified to a study of the prevalence of cervical dysplasia in oral contraceptive users. This decision was made after careful consideration and review by an expert panel and is based on the judgment that the study could not provide a definitive answer to the question in the face of a vast expenditure of time, effort and monies for five years or more.

Several workshops were held by the Branch. One dealt with the topic of vasectomy and included all the contractors currently supported by the Branch in its vasectomy program.

A workshop of oral contraceptives and dietary nutrients took place in the fall of 1973 and a planning session with consultants in February of 1974.

Oral contraceptive use and cervical pathology which included cervical dysplasia, carcinoma in situ and cervical cancer was the topic of a workshop of contractors and outside investigators to assess the current state of knowledge in this area. It brought together eight investigators. Six are currently conducting studies in this country, one has a study in Canada and one in Great Britain.

The Fertility Regulating Methods Evaluation Branch has a serious personnel shortage. It has been carrying forward a highly diversified, substantial contract research program in excess of 50 contracts with a staff of 3 professionals and 2 sub-professionals. In order to sustain the quality of the ongoing program and to expand it further to cover other areas of great concern, additional staff is urgently needed.

Program content and progress of the FRMEB can best be summarized under several specific sub-headings.

General Surveillance:

The vast prospective study of about 18,000 women known as the Walnut Creek

Contraceptive Drug Study continues with emphasis on the enumeration of adverse clinical and sub-clinical effects of low order of magnitude and also the identification of risk factors of such effects drawn from the data as obtained from the Automated Multiphasic Laboratory.

Numerous important findings have emerged from the ongoing analysis of the comprehensive information that has already been collected. Of particular clinical significance is the observed several-fold increase in risk of hypertension in women using oral contraceptives as compared to women using other methods of contraception. The hypertension appears to be reversible, although it is not known whether reversibility is complete. Further, an increase in sub-clinical urinary tract infections or bacteriuria was shown among oral contraceptive users. Numerous biochemical effects as determined by the Automated Multiphasic Laboratory have been noted in oral contraceptive users. The significance of these with respect to eventual clinical complications is as yet undetermined. Only a continuing multi-year follow-up of this population bringing together the observed biochemical changes and the possibly later occurring clinical complications will answer these questions.

Numerous publications have appeared in the scientific literature. Moreover, the currently available information from this project will be presented systematically in three volumes of a monograph. The first volume is completed and is now in print in the Government Printing Office. The second volume is available in draft form and undergoing revision. Volume III is scheduled to be available in draft form by September of 1974.

Evaluation of Carcinogenic Potential

The two case-control studies of the relationship between breast cancer and oral contraceptive use are completed and the final reports will become available during the next few months. The known preliminary results of these two investigations show no increase in risk in cancer of the breast among oral contraceptive users. A small decrease in risk in benign lesions of the breast was observed in oral contraceptive users which is in keeping with the observations of others.

The preliminary results of a study on the association between oral contraceptive use and cervical dysplasia have shown no increase in prevalence associated with the use of oral contraceptive steroids. Another related study of considerable significance deals with the follow-up of women with cervical dysplasia of which some are receiving oral contraceptive steroids and others use other methods of contraception. A slight increase in the rate of progression in severity of cervical dysplasia has been observed after three years of follow-up among the oral contraceptive users.

Interaction of Contraceptive Steroids with Essential Nutrients

This program consists of cross-sectional and longitudinal surveys of nutritive health status in oral contraceptive (OC) users and controls, clinical investigations of interactions of OC with specific nutrients, the consequences of these interactions, and animal studies of the elucidation of the metabolic basis of the interactions of OC with essential nutrients.

The surveys emphasize biochemical nutritional parameters known or suspected to be abnormal in contraceptive users and contain cross-sectional and longitudinal components. Preliminary results have been noted. Findings include increases of white cell count, per cent of abnormal response for 2-hour post-prandial glucose, cholesterol, triglycerides, total lipids, serum iron, and serum vitamin A. There is a significant decrease in the OC group in fasting glucose level, serum albumin, serum alpha globulin, serum folate, whole blood folate and serum B₁₂. The effects of OC on biochemical nutritional parameters appeared largely independent of nutrient intake except for the lowering effect on folate and B₆ levels which may be dependent on intake.

In the future the scope of these projects can be focused on essentially three problems: B₆ deficiency, folate deficiency and zinc deficiency.

In a surprisingly large proportion (>75) of OC users, biochemical changes indicative of deficiency in either one or all three of these essential dietary nutrients occur.

The essential target shall be to establish or rule out causal relationships between abnormal nutritive status of vitamin B₆, folate and zinc and certain adverse medical effects. This may require prospective longitudinal surveillance of a large number of OC users.

An ill-understood, side-effect of the oral contraceptives is an alteration in carbohydrate metabolism with biochemical features similar to maturity-onset diabetes. Estrogen containing oral contraceptives also cause an abnormality of tryptophan metabolism which is like that of vitamin B₆ deficiency and reversed by pyridoxine administration. In view of this research, the effects of pyridoxine were studied on glucose tolerance of oral contraceptive users. Pyridoxine administration produced a marked improvement in the glucose tolerance of women with subclinical Vitamin B₆ deficiency; the others were unaffected. When glucose tolerance was determined before the induction of B₆ deficiency, at the peak of the deficiency and after supplementation with pyridoxine, marked deterioration in glucose tolerance was observed in the oral contraceptive users which was reversed by pyridoxine. There was no significant change in the glucose tolerance of the control subjects, despite the presence of Vitamin B₆ deficiency. It is concluded that vitamin B₆ deficiency and a separate metabolic effect of the contraceptive steroids are required to produce a pyridoxine responsive alteration in carbohydrate metabolism.

A sensitive procedure has been developed for measuring serum pyridoxal phosphate levels. Data obtained show that plasma pyridoxal phosphate levels are reduced with OC use and that this effect can be reversed with pyridoxine supplementation. Erythrocyte glutamic GOT values correlated significantly with plasma pyridoxal phosphate levels.

Trace element balance studies were performed on OCA users and controls under controlled conditions. OCA users had higher BMR and increased nitrogen retention with positive nitrogen balance. There was a marked negative trace mineral balance for zinc. Since dietary zinc intake provided by the average diets of many population groups is lower than the recommended dietary allowances, this may point to a serious public health problem.

Because of the known increase in thromboembolic complications in oral contraceptive users, work of the interaction of contraceptive steroids with Vitamin K absorption, metabolism and utilization in animal models has been supported. In rats prothrombin is more readily generated in females than in males. Further, a polypeptide-type precursor of prothrombin could be altered by estrogens and account for its coagulatory effect. It appears that estrogen permits vitamin K to act more effectively and rapidly.

The effects of Vitamin B complex deficiencies and excess on metabolic hydroxylation of selected steroids were also investigated. The metabolism of mestranol was found to be higher in rats which were fed a thiamin, riboflavin and pyridoxine deficient diet. The mechanism of this effect is to be elucidated in the future course of this project. The finding that metabolic rates of steroids are influenced or determined by dietary nutritional status raises the question whether nutritional states may determine contraceptive efficacy and deficiencies perhaps responsible for contraceptive failures.

In rats the effect of contraceptive steroids resembles those resulting from Vitamin E deficiency. For this reason an investigation was initiated to study the interrelationship between oral contraceptives and Vitamin E. The preliminary results of this project are as yet not conclusive.

Another investigation deals with the effect of oral contraceptive steroids on calcium, phosphorus, magnesium and zinc metabolism with emphasis on bone mineralization. In rats it has been demonstrated that OCA depressed serum zinc levels. The effect is much more due to the estrogen component of the OCA than the progestin component. Calcium, magnesium and phosphorus levels were not affected.

Thrombosis and Thromboembolism

The association and relationships between oral contraceptive use and thrombotic, embolic vascular occlusion was investigated in two retrospective case-control studies. The first project included all occlusions of systemic and pulmonary vessels, except cerebrovascular accidents (stroke). The other study focused on the association of oral contraceptives and stroke.

In the first study a data base of 500 cases of thromboembolism and 500 controls has been accumulated. Preliminary analysis revealed that the overall relative risk of thromboembolism in OC users is about 2.

The crude relative risk for the "idiopathic" groups is 7.2. Almost all of these patients used the low dosage contraceptives, i.e., 100 mcg. or less. This would tend to confirm a recent observation of no reduction in risk of thromboembolism with the low estrogen dosage when examining an "idiopathic series."

The stroke project consisted of 300 patients admitted to the neurologic services of ten collaborating university medical centers and controls. The analysis revealed that the relative risk of cerebral ischemia or thrombosis was about nine times greater for women who use oral contraceptives.

Hypertension was an additional risk factor for development of either thrombotic or hemorrhagic stroke among women using oral contraceptives and those not using these agents. Regular cigarette smoking and a history of symptoms indicative of migraine headache also increased the likelihood of one or the other type of stroke but more information is needed before a definite relationship can be established between these clinical factors and cerebrovascular disease. Oral contraceptives alone, in the absence of smoking, hypertension or migraine, significantly increases the risk of stroke.

Another project is concerned with the development of laboratory tests capable of detecting or predicting intravascular coagulation.

This has resulted in the development and standardization of procedures and methodology of fibrinogen chromatography. Further investigations have pursued the usefulness of fibrinogen chromatography as a screening procedure for assessing the risk of thromboembolic complications in patients on various types of contraceptive therapy.

The data thus far show a finding of greater than 20% fibrinogen complexes indicative of thrombosis in 4.3% of control subjects, in 15% of those on OCA for one month, and in 20% of those on medication for three months. Thereafter, the incidence of abnormality varied between 22% and 28% up to and beyond eight years. The abnormalities in plasma fibrinogen chromatographic behavior were invariably transient and indicative, if the sampling period was sufficiently short, both of the presence of a developing and later, of a resolving, thrombus. The risk factor resulting from use of oral contraceptives, calculated from this data was a 5-6 fold one, which is in line with the epidemiological data.

Hypertension

The substantial risk in hypertension among oral contraceptive users established by longitudinal surveillance has already been mentioned. Further work has attempted to characterize the biochemical effect of various estrogenic and progestogenic compounds on the renin-angiotensin-aldosterone system and on sodium metabolism of normotensive contraceptive users and to study these effects in persons developing hypertension either on the oral contraceptives or on conjugated estrogen replacement therapy (Premarin). A new goal formulated on the basis of past results is to find an OC preparation which lacks the mineralocorticoid effect of the presently available ones.

It has not yet been possible to biochemically differentiate OC users who develop hypertension from those who remain normotensive. In both, OCA cause an increase in renin substrate concentration and renin activity. The effect on plasma renin concentration is less clear.

Roughly 40% of those women who had developed hypertension on estrogenic compounds became normotensive without medication.

About 75% of the hypertensive patients who took spironolactone after they had discontinued estrogenic compounds became normotensive in 1-3 weeks on spironolactone.

Synthetic estrogens, diethylstilbestrol, or the conjugated estrogen, Premarin,

can all cause hypertension. The cause appears to be a result of a mild hypermineralocorticoid state produced by the medication coupled with the absence of the antiminerlocorticoid effect of natural progesterone (because it is suppressed).

Pharmacology of Contraceptive Steroids

Although the oral contraceptive steroids have been in use for over a decade and a number of side effects have been demonstrated in clinical and epidemiological studies, very little is known of the metabolism and metabolic effects of these compounds in women or in animal models used in long-term toxicity testing. Such information is necessary to determine the mechanisms involved in the observed side effects and to identify appropriate animal species for predicting toxic effects in man.

In a study of the metabolism of norethindrone and mestranol in women, it has been demonstrated that the administered drug is very rapidly converted to metabolites some of which circulate in the blood for relatively long periods of time. The identity and half-life of these metabolites is being determined and the possibility that some metabolic products may accumulate in the blood in chronic users of contraceptives is being investigated. In another study, the metabolic products of mestranol are being studied in urine from women who are long-term users of OCs containing mestranol, and work is in progress to develop radioimmunoassays for measuring small amounts of mestranol and ethinyl estradiol in blood.

Several of the animal studies in progress emphasize work in the beagle dog, a species in which chronic toxicity studies of contraceptive steroids are required by the FDA. The effects of medroxyprogesterone acetate are being investigated in a longitudinal study of pathologic changes in the beagle mammary gland and uterus; particular attention is being devoted to morphologic and biochemical changes associated with the development of mammary lesions. In another study, assays have been developed to measure a number of endogenous hormones in beagles and the amounts of estrogen, progesterone, adrenal corticoids and LH in blood have been determined in the normal estrous cycle and during pregnancy as a preliminary to determining the effects of chronic treatment with medroxyprogesterone acetate on levels of these and other hormones. In a project conducted at the Armed Forces Institute of Pathology, tissues from beagles, monkeys, and rats treated with contraceptive steroids in long-term toxicity studies have been obtained from a number of pharmaceutical companies for pathologic evaluation, particularly of the beagle mammary lesions.

While toxicity tests are now required in beagles and monkeys, it is possible that other species may be more suitable. Several projects are concerned with the metabolism of contraceptive steroids in the baboon. One project has developed a surgical technique which makes it possible to study the effects of estrogens and progestins on liver function and the composition of bile in the baboon, and one is comparing the effects of estrogens and progestins on carbohydrate and lipid metabolism in baboons, beagles and women. A large project is concerned with the metabolism and biochemical and biological effects of a series of progestins in a variety of target tissues in rodents, dogs, monkeys,

and later in women. Another equally broad project is accumulating extensive data to provide comparative profiles of biochemical effects of three progestins, alone and in combination with mestranol, in rats, mice guinea pigs, and rabbits. While much of the data in this latter study has been negative in character, such information is much needed and interesting effects have been demonstrated in the suppression of immune responsiveness by estrogen-progestin combinations. Preliminary results also suggest that these combinations have no effect on cancer growth and metastasis in three test systems, and significant species differences between rats and mice have been demonstrated in effects of mestranol on levels of various lipid fractions in blood and tissues.

Vasectomy.

Vasectomy is an accepted and effective method of fertility control. There is little information on the possible long-term medical consequences of this procedure.

A specific program to develop this needed information was initiated in FY 72 and FY 73. It attempts to define morphological, endocrine, and immunologic sequelae of the procedure and to provide information on the medical status of vasectomized men. Early results are now beginning to become available from some of these studies.

Vasectomy techniques in animals result in an accumulation of sperm in the vas and epididymis and ultimately rupture of the walls of these structures. These phenomena are important as a possible site of initiation of an immune response to sperm and because of possible pressure effects on testicular and epididymal function. Micropuncture techniques are used to study intratubular hydrostatic pressure in the seminiferous tubules, caput and cauda epididymis of the hamster before and after vasectomy. In animals in which no ruptures occur markedly increased pressure in the tubules of the distal cauda and epididymal vas deferens is noted while pressure in the seminiferous tubules and caput is approximately normal. In animals in which rupture has occurred and large granulomas are present, pressures are in the normal range. The mechanisms by which a pressure differential is maintained in the epididymis are not clear.

Approximately 50% of vasectomized men demonstrate circulating antibodies to sperm, presumably as a result of exposure of sperm to the immune system by spillage at the time of operation, rupture of the epididymis or vas, or from damage to the blood-testis barrier. Little is known of the specific antigens of sperm to which antibodies are formed. In a basic study of sperm immunology mice have been immunized against antigens on the surface of mouse sperm determined by genetic alleles at the T locus. Antisera against sperm carrying any of four recessive alleles react only with sperm of the immunizing type and no cross-reactions within this group of alleles have been detected. Some other alleles may share some antigenic specificities. It has been demonstrated by syngeneic immunization that antigens determined by the T locus can function as autoantigens.

A presumably nuclear autoantigen has been obtained from human sperm heads swollen by treatment with dithiothreitol and trypsin and the antigen has been solubilized, isolated and purified. It is a strongly basic protein of low molecular weight. Further chemical characterization of the antigen is in progress. Antibodies to this antigen are not identical with those causing sperm agglutination and sperm immobilization reactions.

Studies of men before and after vasectomy have utilized immunofluorescence techniques to detect circulating antibodies to sperm antigens. The presence of antibodies has been observed reacting with several regions of the sperm head of 50% of 80 men before vasectomy. Titers of these antibodies increased at two months after the operation but showed little change at nine months. A second type of antibody, reacting with the sperm tail and occasionally with discrete areas of acrosome, was rarely found before vasectomy but occurred in 25% of patients at two months and 50% at nine months after the operation. Immunoglobulin classes of these antibodies have been determined and occur as IgG and IgM.

Sera from vasectomized men are tested for antibodies to steroid-producing cells of the adrenal and testis by immunofluorescence and immunoperoxidase techniques. In very preliminary results, no reactions with steroid producing cells of the testis have been demonstrated in these sera. Antibodies to testicular tissue have been found in vasectomized rabbits and the cellular source of the antigens is being investigated.

Two longitudinal studies of the immunologic status of vasectomized men have thus far shown no abnormalities, however, considerable variability has been seen in some measures of cell-mediated immunity to sperm antigens. This may be inherent in the nature of the tests or peculiar to genital antigens, or may be a result of vasectomy.

A longitudinal study of plasma levels of steroid and gonadotrophic hormones in vasectomized men has also been under way. There have been no adverse changes in any hormone for periods up to one year after vasectomy. Data based on small numbers of subjects suggest that plasma testosterone levels may be significantly higher a year after vasectomy than before the operation and during the early post-operative months. This difference is unexplained but may be due to unusually low pre-operative values, perhaps because of psychological stress.

Plans For New Programs

The beginning of a comprehensive investigation of the problem of hypertension in response to oral contraceptive steroids will get under way in FY 75. We will include the study of risk factors predictive of the hypertensive response, the reversibility after discontinuation of oral contraceptives and the pathophysiology of it.

Also under consideration are studies aimed at the evaluation of reproductive performance after induced abortions. Of particular concern is the alleged increase in premature labor among subsequent pregnancies in women who previously underwent one or more induced abortions.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Contraceptive Drug Study
Contractor: Kaiser Foundation Research Institute, Walnut Creek, Calif.
Money Allocated: \$766,401 (FY 1974 Funds)

Objectives: The objective of this program is to evaluate a variety of biochemical and medical parameters as related to contraceptive use in women who are members of the Kaiser Permanente Medical Care Program in Walnut Creek, California. The principal tool for accomplishing this objective is an automated multiphasic laboratory examination in which more than 17,000 women have been studied at least once. The long-range objective is to make longitudinal observations on various components of the automated multiphasic examination and to relate these to morbidity and mortality in the population.

Significance to Biomedical Research and Program of the Institute: This is a broad ranging program of surveillance of the possible effects of the use of various contraceptive methods with particular emphasis on the oral contraceptives. The particular value is the ability to monitor a large population in considerable depth and to be able to relate biochemical to clinical effects. It is relevant to the Center for Population Research program to monitor medical effects of oral contraceptives.

Major Findings: The findings from this project are documented in numerous publications and will shortly appear in three volumes of a monograph series based on the cross-sectional data from this project. Volume 1 of the monograph has been completed; Volume 2 is undergoing revision and Volume 3 will be available in draft form by September 1974. Of particular significance as well as concern is the recent observation from the follow-up of oral contraceptive users in this project for 1-1/2 years of a six-fold increase in hypertension as compared to users of other methods of contraception. Also, an increase in sub-clinical bacteriuria was recently reported from this project in oral contraceptive users. Effects of oral contraceptives on glucose tolerance and of other estrogens on glucose absorption have been documented and also an increase in serum triglycerides among OC users.

Proposed Course: The population of about 17,000 women enrolled in the Contraceptive Drug Study is followed by interim questionnaire annually. In addition, laboratory examinations using the automated multiphasic laboratory are obtained on samples of women with the selection process so that every woman will be subjected to the automated multiphasic laboratory examination once every three years. All sources of medical information within the Kaiser system about the study participants regarding the intercurrent medical events requiring out-patient visits or in-patient care are abstracted to monitor medical events occurring in users of different forms of contraception.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Steroid Contraception on Cervical Dysplasia
Contractor: University of California, Los Angeles
Money Allocated: \$220,000 - (FY 1973 Funds)

Objectives: The project is to evaluate the effects of steroid contraception on cervical cancer by studying dysplasia of the cervix. It consists of a follow-up of somewhere around 300-400 patients enrolled during a period of 1967 through 1972 and drawn from Family Planning Clinics of the Los Angeles County Health Department. The enrollment is limited to women with evidence of cervical dysplasia as defined by the principal investigator. Some of these women are using oral contraceptives, others make use of other means of contraception. The follow-up of this population is intended to provide information on the rate of progression or the lack of it of cervical dysplasia by contraceptive status.

Major Findings: The important finding, undoubtedly, from this project is the observation of a slight increase in the rate of progression of cervical dysplasia among oral contraceptive users after about 2-1/2 to 3 years follow-up. This finding has persisted through the additional data made available during the past year. While this is an important observation there is still to be resolved the problem of the considerable loss rate from follow-up of a large portion of the study population. Since the observed differences are small it is absolutely crucial that the further analysis take into consideration the losses from observation and the possible bias which may have resulted therefrom. It is conceivable that the loss rate is so high that further follow-up of this population would not be productive.

Significance to Biomedical Research and the Program of the Institute: This program relates obviously to the issue as to whether oral contraceptives may have a carcinogenic effect on the human cervix. Women with cervical dysplasia, at least in the eyes of some investigators, are considered to be at risk of developing carcinoma of the cervix. A careful follow-up of this nature by a competent investigator undoubtedly could add significantly to our information and understanding.

Proposed Course: Clearly the thorough and complete evaluation of the data base is the next order of business in order to ascertain whether the findings noted are genuine and not spurious because of loss and bias thereof. The resolution of this question will determine the future course. If the findings hold up we propose continuing follow-up through careful cytological assessment at intervals of this population.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: A Five-Year Prospective Study of Abnormal Cervical Cytology
Contractor: Temple University, Institute for Survey Research
Money Allocated: \$392,215 (FY 1974 Funds)

Objectives: This project was originally conceived as a five-year prospective study of the possible relationship between changes in cervical cytology and the use of various methods of contraception. A careful review of this project resulted in a substantial change in its scope, as follows: The project was limited to an evaluation of the prevalence of cervical dysplasia and carcinoma in situ of the cervix among oral contraceptive users and comparing the prevalence to that of similar women who use other methods of contraception. At the time this decision was made the project had enrolled about 27,000 women and the problem under study is therefore limited to this population.

Significance to Biochemical Research and Program of the Institute: Oral contraceptive agents are known to have a stimulatory effect on the tissues of the uterine cervix. The project population consists of three major ethnic groups drawn from different parts of this country. It provides an opportunity to review prevalence rates of cervical cytology in different ethnic groups by oral contraceptive use which is relevant to pursue the question of the long-range safety of oral contraceptives.

Proposed Course: With the change in objectives, enrollment into this project was terminated at 27,000 enrollees and preparations for the analysis of this data began. This included follow-up on incomplete or uncertain cytological status and completion of other unknown items of information relevant to the analysis of these data.

Major Findings: The report of the analysis is expected in the fall of 1974. Preliminary analysis reveals no difference in risk of cervical dysplasia by oral contraceptive user status. However, this does not as yet take into consideration the duration of exposure.

NICHD ANNUAL REPORT
July 1, 1973 through June 22, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: An Epidemiologic Case-Control Study of Thromboembolism
Related to the Use of Oral Contraceptives
Contractor: Johns Hopkins University - School of Hygiene & Public
Health
Money Allocated: \$30,200 (FY 1973 Funds)

Objectives: The purposes of this study were: 1) to investigate the association between oral contraceptive use and thromboembolism in order to define further the risks of developing these phenomena among users; 2) to provide a continuing surveillance of the risks of thromboembolic disease associated with the usage of oral contraceptives and to monitor adverse reactions to new formulations as they come into wider use; 3) to define further any possible predisposing factors that may increase the risk of developing thromboembolic disease in oral contraceptive users; 4) to determine if there is a difference in the relative risk of developing thromboembolic disease associated with different formulations of oral contraceptives e.g., sequentials and non-sequential; and to determine if a dose-response relationship occurs with increasing amounts of estrogen; and 5) to investigate further the reported proportional lesser incidence of thromboembolism in persons of blood group O.

Major Findings: 1) All operational procedures and preliminary analysis have been completed. The accumulated data base consist of 500 cases of thromboembolism and 1000 controls. 89 of the cases were classified as idiopathic. The criteria for this classification were the absence of known pre-disposing or precipitating causes of thromboembolism, other than oral contraceptives.

2) The overall relative risk that thromboembolism was due to oral contraceptives for the entire sample of 500 cases and their respective controls is 1.9-2.5

3) The crude relative risk for the idiopathic group is 7.2. Almost all of these patients used the low dosage contraceptives, i.e., 100 mcg. or less. This would tend to confirm the recent observation by Jick et al of no reduced risk of thromboembolism with the low disage pills, when examining an "idiopathic series".

4) A dose response could however be demonstrated for the "non-idiopathic" cases. The dose response relationship, however appears complex and not linear. The risk associated with the 50 mcg estrogen pill was lower than that of the 100 mcg pill. Those with intermediate estrogen content appeared to have the lowest risk.

5) Preliminary analysis suggests that no particular predisposing conditions will be uncovered by this study which might be used to predict (and thus

label as contraindications) higher risk of thromboembolism should these women use the oral contraceptives.

Significance to Biomedical Research and Program of the Institute: Several studies have now established an association between oral contraceptive usage and the development of thromboembolic disease. Questions as yet unanswered concern the relative risk of developing thromboembolic disease as related to usage of different formulations of oral contraceptives containing various amount of estrogenic compound or a progesterone. In addition, it is still unclear which medical conditions or predisposing factors increase the risk of developing thromboembolic disease among oral contraceptive users. Studies supported by this contract should provide essential information to establish or rule out potential contraindications to the use of oral contraceptives. It should also help to determine which formulations of these agents present the greatest hazards to users. This study is important, well-designed, and is being carried out by an experienced group of investigators. The significance of the study is re-emphasized by recent articles questioning the association of oral contraceptives and thromboembolism.

Proposed Course: This is limited to final data analysis and reporting the results. Final data analysis will include:

- a. Crosstabulations and applications of statistical models to control for possible confounding effects of other variables on the association of thromboembolism and female hormone use. There are at least five different models now in use for controlling confounding effects in retrospective studies. Seigel and Greenhouse have recently presented two models and Miettinen another based on discriminant functions. Two other models (linear and logistic) using regression methods where hormone use is taken as the outcome variable are also competitors. In view of the close scrutiny that other similar studies have been subjected to, application of these methods would be desirable to confirm and strengthen the basic study findings. Additionally, the data provide an ideal basis for comparison of the five adjustment techniques and we anticipate that a publication detailing the comparison would be forthcoming.
- b. Analyses of our large pool of controls to look for other potential associations and to assess their effect on the analysis of the main study. It is contemplated to examine the relationship of OC use of biliary tract disease, an association reported by Jick recently.
- c. Further analysis of dosage and relative risk; progestagen content and type of estrogen/progestagen to risk.
- d. Validity/consistency checks on the interviews and chart abstracts as well as location of "missed" cases will continue.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: A Collaborative Study of Oral Contraception and Cerebrovascular Disease
Contractor: Duke University Medical Center
Money Allocated: \$25,507 (FY 1973 Funds)

Objectives: The objectives of this contract were to determine whether the incidence of stroke in young women is increased by use of oral contraceptives, and if so to identify characteristics that are especially related.

Description of Project - Summary: The Neurologic Services of ten university medical centers have identified all young women admitted to their hospitals with definite or suspected cerebrovascular disease of any type. Four of the participating hospitals also identify patients admitted to each of the community hospitals in their metropolitan areas (Miami, St. Louis, Baltimore, and Atlanta). An estimated 200 patients with cerebrovascular disease are submitted for study each year. Clinical data on each of these patients are abstracted from the hospital and physician's records. The frequency of usage of oral contraception in these patients was compared with that found in two selected control samples: 1) women hospitalized for other diseases and 2) women representing a "healthy" population residing in the same neighborhood as the patients selected for the study. Information regarding pill usage was obtained from the patient and control subjects by personal interviews carried out in their homes.

Major Accomplishments and Findings: 1) The current use of oral contraceptives was considerably increased in women with thrombotic strokes as compared with their controls and somewhat increased in women with hemorrhagic strokes. The relative risk of cerebral ischemia or thrombosis was estimated to be about nine times greater for women who use oral contraceptives than for those who do not.

2) Hypertension was found to be an additional risk factor for development of either thrombotic or hemorrhagic stroke among women using oral contraceptives as well as those not using these hormonal agents. Regular cigarette smoking and a history of symptoms indicative of migraine headache also increased the likelihood of one or the other type of stroke but more information is needed before a definite relationship can be established between these clinical factors and cerebrovascular disease.

3) Oral contraceptives alone, in the absence of smoking, hypertension or migraine, significantly increases the risk of stroke.

Significance to Biomedical Research and Program of the Institute: The study is an extension of previous research on thromboembolism and oral contraceptive use in which very few stroke cases were included and is a part of our program to monitor the significant effects on health of currently used contraceptives.

Proposed Course: The project is completed.

NICHD ANNUAL REPORT
July 1, 1973 through June 22, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Contraceptives on Blood Coagulation
Contractor : Washington University School of Medicine, St. Louis, Mo.
(P.I.=Anthony, P. Fletcher, M.D., Associate Professor
of Medicine)
Money Allocated: \$98,600 (FY 1974 Funds)

Objectives: The objectives of this contract are: 1) to demonstrate the clinical usefulness of fibrinogen chromatography as a screening procedure for assessing the risk of thromboembolic complications in patients on various types of contraceptive therapy; 2) establish the scientific basis and significance underlying the development, appearance, disappearance, and reappearance of abnormal Fibrinogen Chromatograms; 3) further develop, improve and standardize the new diagnostic method of fibrinogen chromatography.

Major Findings: The principal investigator has standardized and published his procedure of fibrinogen chromatography. He has succeeded to remove most or all doubts about the validity of his method. The major postulates originating from the project have been continued. It has become rather generally accepted that fibrinogen chromatography is a sensitive and specific test reflecting ongoing intravascular fibrin formation even at a subclinical level, without manifest thrombosis. The results of automating the procedure have been to substantially increase methodological reproducibility, increase speed of the procedure performance, provide objective interpretation of results and finally, to reduce costs.

Fibrinogen Chromatography in Users of Oral Contraceptives: Initial application for assessment of utility and feasibility - these were rather extensive pilot studies carried out in St. Louis (in collaboration with Dr. Robert Burstein, Department of Obstetrics and Gynecology, Washington University School of Medicine). A rigorous epidemiologic design and operational procedure for this work apparently had never been developed, at least it has never been precisely formulated in the preceding contract periods. Abnormality in the plasma fibrinogen chromatographic findings, that is, finding of greater than 20% fibrinogen complexes indicative of thrombosis, was found in 4.3% of control subjects, in 15% of those on medication for one month, in 20% of those on medication for three months. Thereafter, the percentage incidence of abnormality with this examination varied between 22 and 28% for all time periods up to, and beyond, eight years. The subjects' age was not a significant factor in this analysis. It is emphasized that the abnormalities in plasma fibrinogen chromatographic behavior were invariably transient and indicative, if the sampling period was sufficiently short, both of the presence of a developing and later, of a resolving, thrombus. The risk factor resulting from use of oral contraceptives, calculated from this data was a 5-6 fold one, a conclusion in line with the epidemiological data. Also, the data shows a definite association between the findings of abnormal chromatographic results and complaints by the patient of symptoms suggestive of, but not

diagnostic of, thromboembolic vascular disease (p. 0.01).

In order to overcome epidemiological criticism against methodological design deficiencies of the studies team which these results emerged an epidemiological rigorous prospective blind study was initiated. Its specific purpose was to provide conclusive evidence as to the clinical value and usefulness of fibrinogen chromatography for surveillance of contraceptive users. This collaborative project comprising longitudinal surveillance of Demulen users (a "low estrogen" oral contraceptive) versus concurrently studied control unmedicated subjects was set up in Kansas City with Dr. Robert Newman. Numerous operational difficulties were encountered in the collection of samples, their storage and transport to St. Louis. As a consequence final evaluation of the investigation is not available. These operational difficulties have now been overcome. Preliminary results available confirm the original St. Louis studies with respect to the incidence of plasma fibrinogen chromatographic abnormality following OC usage.

Significance to Biomedical Research and Program of the Institute: The development of a laboratory test capable to detect or predict intravascular coagulation is urgently needed in several fields of clinical and investigative medicine. It is of particular importance in contraceptive management, since unexpected thromboembolism appears to be a major well-documented side effect of contraceptive steroids.

Proposed Future Course: 1) Complete the clinical studies with a prospective epidemiologic design, presently in progress with Dr. Robert Newman, St. Lukes Hospital, Kansas City.

2) The collaborative studies on high risk patients with correlation of I²⁵⁵ labeled fibrinogen uptake are to be continued with the objective to further refine clinical assessments, diagnostic and prognostic potentials of fibrinogen chromatography in contraceptive users.

Continue methodological development and refinement of fibrinogen chromatography. This work is to be mainly directed towards achieving higher resolution of the eluted fibrinogen complexes, with the aim of separating those patients with abnormal chromatograms at high risk of developing clinically overt thrombosis from those who have also abnormal chromatograms but are at lesser or low risk of clinical overt disease.

The scientific basis and significance underlying the development, appearance, disappearance, and reappearance of abnormal fibrinogen chromatograms will be established by:

- (a) Plasma clearance of various fibrinogen derivatives;
- (b) Characterization of fibrinogen chain linkage in chromatographic fractions which are caused by Factor XIII and indicative of actual thrombus formation;
- (c) Correlations between abnormal patterns obtained on Fibrinogen

chromatography of plasma and fibrinopeptide A content of plasma.

NICHD ANNUAL REPORT
July 1, 1973 through June 29, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Effect of Oral Contraceptives and Conjugated Estrogens on Renin Activity and Aldosterone
Contractor: Loma Linda University, Loma Linda, California
(P.I. Milton G. Crane, M.D.)
Money Allocated: \$28,077 (FY 1973 Funds)

Objectives: The investigations conducted under this project are aimed towards three principal goals. Firstly, to characterize the biochemical effect of various estrogenic and progestogenic compounds on the renin-angiotensin-aldosterone system as well as on sodium metabolism of normotensive contraceptive users. The second goal is to study these effects in persons developing hypertension either in young women on the oral contraceptive pill or in menopausal women receiving conjugated estrogen replacement therapy (Premarin). A new goal formulated on the basis of past results is to find an OCA preparation which lacks the mineralocorticoid effect of the presently available ones.

Major Findings: Estrogenic compounds in normal subjects exhibited a stimulatory effect on the renin-angiotensin system with the production of a mild hyperaldosterone state and sodium retention. This is associated with a marked increase in renin substrate with a slight increase in renin activity and aldosterone excretion rate under basal conditions. The responsiveness of the renin-angiotensin system to sodium restriction and standing is accentuated. Plasma levels of aldosterone, however, were unaffected. The administration of the estrogen was associated with about a 200 mEq increase in exchangeable sodium. The addition of 25 mg to spironolactone with the usual dose of ethinyl estradiol was insufficient to counteract the sodium-retaining properties of the estrogen. A larger dose would appear to be needed.

Two gestagens that were studied appear to cause sodium retention and suppress aldosterone excretion rate by a direct mineralocorticoid-like effect. Some gestagens have what appears to be an estrogenic effect with an increase in substrate concentration.

Natural progesterone, on the other hand, causes a negative sodium balance with a secondary increase in renin activity and aldosterone excretion rate.

A study of the data obtained from the patients who had developed hypertension after starting an estrogenic compound indicated the following points: When the estrogenic compound was discontinued plasma renin activity and aldosterone excretion rate decreased generally, and the exchangeable sodium decreased in the majority of the patients. Some of the patients continued to have a slight elevation in exchangeable sodium one month after the medication was discontinued.

Roughly 40% of those who had developed hypertension on estrogenic compounds became normotensive without medication. It requires up to 12 months after the medication was discontinued before the patients remained normotensive

spironolactone administration. About 75% of the hypertensive patients who took spironolactone after they had discontinued estrogenic compounds became normotensive in 1-3 weeks on spironolactone.

Synthetic estrogens, diethylstilbestrol, or the conjugated estrogen, Premarin, can all cause hypertension. The cause appears to be a result of a mild hypermineralocorticoid state produced by the medication coupled with the absence of the antiminerlocorticoid effect of natural progesterone (because it is suppressed).

Significance to Biomedical Research and Program of the Institute: The scientific value of the proposed work lies in its promise to clarify pathophysiological relationships between steroid metabolism, electrolyte balance and hypertension. This knowledge is relevant to the program of the Center in order to assess risk and consequences of contraceptive induced hypertension and possibly devise an approach to its therapy and/or prevention.

Proposed Course: Four lines of work are contemplated for the next contract period, proceeding towards the following objectives: (1) To find more patients with hypertension who date the onset of their elevation in blood pressure after starting an estrogenic compound or an oral contraceptive agent, (2) to continue observations of exchangeable sodium, renin substrate, renin activity, and aldosterone levels in urine and blood in those patients who had developed hypertension on estrogenic compounds in an effort to determine the pathological physiology of this type of hypertension, (3) to find an orally effective gestational agent that has sodium diuretic properties similar to natural progesterone, and (4) to evaluate the effect on the renin-angiotensin-sodium system of a larger dose of spironolactone in combination with an estrogen and the effect of one or two gestational agents that promise to have sodium diuretic properties. The latter will include a study of the effect of these on exchangeable sodium, renin activity, and aldosterone concentration in plasma and urine.

The effect of a combination of ethinyl estradiol with a 100 mg per day dose of spironolactone and the effect of a new progestational agent, Norgestrol (Wyeth), on the renin angiotensin-aldosterone system and exchangeable sodium or normal subjects are also to be investigated.

In the near future the FRMEB branch intends to issue a Request for Proposal on problems investigated in this project. The P.I. (Dr. Crane will therefore have to compete with all proposals received). For this reason the future fate of the project and its length of duration are uncertain.

The RFP will be based upon the following considerations and rationals besides thromboembolism, hypertension is the major adverse medical effect of oral contraceptives. Its etiology, and pathogenic mechanism are unknown. According to one hypothesis estrogens cause an increase in renin substrate concentration in normotensive and hypertensive subjects. An increased concentration of renin substrate effects a lowering of plasma renin concentration in normotensive but not hypertensives (Defective Feedback Mechanism!)

Apart from deficiencies in the knowledge of pathogenesis, the epidemiologic

characteristics, consequences, and the magnitude of the public health problem resulting from OCA induced hypertension are also not understood.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Endogenous and Exogenous Sex Steroids and Nutritional Status
Contractor: The University of Texas Medical Branch, Galveston, Texas
Money Allocated: \$114,600 (FY 1973 Funds)
60,000 (FY 1974 Funds)

Objectives: This is one of three ongoing projects in the program of the FRMEB concerned with the broad characterization of the effects of OC's on the metabolism, requirements and status of micronutrients. The research program is designed to utilize cross-sectional and longitudinal approaches to elucidate the interrelationships between exogenous female sex hormones and a variety of nutritional-metabolic parameters. The investigative work has been aimed towards three circumscribed and specific objectives. (1) A baseline of the normal variations on nutritional status occurring during the menstrual and contraceptive treatment cycle will be established. (2) The effects of oral contraceptives on nutritional status of contraceptive users will be assessed in a cross-sectional comprehensive nutrition survey. (3) The time dependence of oral contraceptive-induced changes in nutritional status will be characterized.

Major Findings: Food intake data suggested that OCA users ingest less riboflavin, vitamin C, vitamin A, vitamin B₁₂ and calcium. OCA use did not appear to alter hemoglobin, hematocrit, total serum proteins, serum albumin or urinary excretion of thiamin or riboflavin. Changes were noted in the α and β -globulins in serum. Serum levels of copper, cholesterol and triglycerides were higher in OCA users. Serum iron levels also fell but could be reversed with iron supplements. Serum vitamin C levels fell during the menstrual cycle which emphasized care as to the time blood samples are obtained.

The baseline study of normal variation on nutritional status occurring during the menstrual and contraceptive treatment cycle has been completed and yielded some significant variation. Final analysis of all these data has not yet been reported. Studies directed towards achieving objective 2, are also completed and await final analysis studies directed towards achieving objective 3 are still in progress.

Significance to Biomedical Research and the Program of the Institute: The investigative work carried out in this project consists of applied rather than basic research and should provide information on possible nutritional consequences and interrelations of contraceptive therapy. The research program is similar in content to two other projects (72-6-013-Prasad and 72-6-019-Smith) and is highly relevant to the objectives of the Institute carried out in the FRMEB, CPR.

Proposed Future Course: The time dependence of oral contraceptive induced changes in nutritional status will be characterized. Emphasis will be to

establish clinical correlation and clinical consequences of the abnormal biochemical-nutritional parameters which have been observed and established during the past contract period.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Interaction of Contraceptive Steroids with Essential Dietary Nutrients
Contractor: Wayne State University School of Medicine
Money Allocated: \$77,550 (FY 1973 Funds)
\$70,000 (FY 1974 Funds)

Objectives: The purpose of the epidemiological clinical study is to characterize nutritional profiles of selected and well-defined sub-populations of the Detroit area belonging to different socio-economic levels and users of oral contraceptives. Specifically, status of trace elements and vitamins will be investigated in this study. A correlation between the abnormal nutritional parameters and use of oral contraceptive pills will be sought by including proper controls of similar socio-economic backgrounds who are not using any oral contraceptives. Nutritional status of all subjects will be determined by proper physical examination and detailed dietary history. This will provide a basis for proper evaluation of changes due to use of oral contraceptives per se and interaction between the pills and poor nutrition on various abnormal parameters can be studied.

Major Findings: OCA cause serum copper levels to increase markedly, zinc serum levels to fall somewhat, serum iron levels to remain unchanged, and TIBC to go up somewhat. Erythrocyte levels of zinc and copper were not altered. Plasma levels of calcium and magnesium were not influenced by OCA use. Serum vitamin C levels appeared to decrease while serum vitamin A levels increased with OCA use. Serum cholesterol levels appeared to remain unchanged. Erythrocyte folacin levels were lower in OCA users (222 ng/ml RBC vs 155 ng). Serum folacin levels were not markedly changed (6.1 ng/ml vs 5.3; NOCA vs OCA).

Significance to Biomedical Research and Program of the Institute: The scientific merit of this project is in its broad coverage of most of the important nutritional factors possibly interacting with oral contraceptive drugs. This study will produce useful information on the effects of contraceptives under the conditions of everyday life, against the background of a good dietary history in two socio-economic classes. The nutrients to be determined are well chosen. The dietary histories will be of value not only in the context of the contraceptive actions but also to nutrition in general. From a nutritional point of view, it is essential to assess any pharmacological effect against the background of dietary history and the conditions of every day life. Even the most sophisticated studies on a metabolic ward have to be extrapolated to conditions outside the ward.

Proposed Future Course: The time dependence of oral contraceptive induced changes in nutritional status will be characterized. Emphasis will be to establish clinical correlations and clinical consequences of the abnormal biochemical-nutritional parameters which have been observed and established during the past contract period. A special feature of this project is to elucidate health significance of subclinical zinc and possibly calcium deficiency.

NICHD ANNUAL REPORT

July 1, 1973 through June 30, 1974

Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Relationships of Contraceptive Steroids to Nutritive State
Contractor: Tulane University
Money Allocated: \$110,916 (FY 1973 Funds)
\$110,000 (FY 1974 Funds)

Objectives: This project is based upon the hypothesis, that since contraceptive steroids produce physiologically a "pseudo pregnancy" state, nutritional complications of pregnancy may develop in persons using these steroids. The general purpose of this proposal is to examine the nutritional status of a number of individuals, who are potentially at high risk nutritionally and attending a family planning clinic. Some will be taking contraceptive steroids and others will serve as controls. It is felt that if contraceptive steroids create nutritional stresses, these can be most easily identified by studying women who received CS through family planning clinics. The specific objectives of this project are to examine the nutritional status of 50 new and 100 long-time users of oral contraceptives who are potentially at high risk nutritionally. Results shall be compared with two appropriately matched control groups who use other methods of contraception.

Major Findings: Investigations in progress have revealed lower hemoglobin, hematocrit, serum iron and TIBC values in non OCA users. Total lipids, cholesterol and triglycerides were increased in OCA users. Vitamin E levels in serum were somewhat higher in OCA users. Serum vitamin A levels were not affected while serum vitamin C levels were low in both OCA users and non-users. The vitamin C levels, however, appeared to reflect the nutritional status of the population under study in Louisiana. Serum proteins and prothrombin times were not affected with OCA use, but some increase in glucose tolerance time was observed in OCA users. A marked incidence of low serum and erythrocyte folacin levels was observed in OCA users. Vitamin B₁₂ fell also in OCA users.

Significance to Biomedical Research and Program of the Institute: This project promises to provide detailed information on the broad nutritional profile of young women beginning contraceptive therapy immediately after pregnancy and of long time contraceptive users. The characteristics of the population to be studied offers several unique advantages: Past experience indicates a high prevalence of anemia, marginal or low serum vitamin A levels and possible folic acid deficiency. The experimental design of the project appears scientifically sound and clearly elaborated. It should provide a broad spectrum of data which characterize the influence of contraceptive steroids on the nutritional health status in a population from a low socioeconomic level.

Proposed Future Course: The time dependence of oral contraceptive induced changes in nutritional status will be characterized. Emphasis will be to establish correlations clinical correlations and clinical consequences of abnormal biochemical-nutritional parameters, which have been observed

during the past contract period. A special feature of this project is the focus on young women of low socioeconomic level who use contraception immediately after pregnancy. This will allow to assess additive effects of pregnancy and contraception on health status.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Evaluation of Effects of Oral Contraceptives on Vitamin B₆ Nutrition
Contractor: University of Wisconsin Medical School
Money Allocated: \$26,300 (FY 1973 Funds)
\$35,000 (FY 1974 Funds)

Objectives: The objectives of this study are to determine if the use of oral contraceptives enhances the rate of induction of vitamin B₆ deficiency in women on a defined intake of vitamin B₆ and folic acid, and to determine accurately the amount of vitamin B₆ required to correct the metabolic alterations observed. A further objective, developed during the course of this project, was to characterize the influence of OCA induced alterations in B₆ status and/or tryptophan metabolism on carbohydrate metabolism, e.g. diabetogenic effects of oral contraceptives.

Major Findings: This has possibly been the most productive contract in the program; Oral Contraceptives and Essential Nutrients. Excellent progress has been made. All the original objectives have been achieved and increasingly significant new ones have been developed.

The estrogen component of OCA appears to be the cause of the main effects on altered vitamin B₆ and tryptophan metabolism. The effects can be reversed with supplements of vitamin B₆. The effects of OCA on women receiving controlled intakes of vitamin B₆ were investigated. Under these conditions, OCA did not effect serum folacin levels, electroencephalogram measurements, or 4-pyridoxic acid excretion. Kynurenine, hydroxykynurenine, and xanthurenic acid excretions were higher with OCA use. This could be reversed with vitamin B₆ supplementation (2.0 mg vitamin B₆ usually adequate). Plasma pyridoxal phosphate levels fell initially in the study and then returned to normal, except subjects on OCA appeared to have levels below those observed for the non-OCA users. The methionine load-cystathionine excretion test did not indicate any difference in vitamin B₆ requirement with the use of OCA, while the tryptophan load-xanthurenic acid⁶ excretion test did suggest an increased requirement for vitamin B₆ with OCA use. Erythrocyte GPT and GOT activities did not appear to be influenced by the OCA user. Dr. Brown suggested that the OCA effects on vitamin B₆ are the result of OCA stimulation of the pituitary-adrenal axis which results in an increased corticosteroid production, which in turn causes an increase in tryptophan oxygenase activity. Their studies did not suggest any change in vitamin B₆ uptake or total body vitamin B₆ with OCA use. Rather, the use of OCA probably alters the activities of certain enzymes in a manner which resembles the changes seen in vitamin B₆ deficiency.

Dr. Rose, the new P.I., is characterizing the effects of vitamin B₆ on the oral glucose tolerance of OCA users. He observed an impaired glucose tolerance in vitamin B₆-deficient oral contraceptive users. This abnormality was reversed by pyridoxine. Such an altered glucose tolerance test was not observed in vitamin B₆-deficient non-OCA users.

Significance to Biomedical Research and Program of the Institute: The diabetogenic effects of oral contraceptives is of great scientific interest and public health significance. The association of these alterations in carbohydrate metabolism with OCA induced changes in vitamin B₆ status and tryptophan metabolism is a completely unexpected and exciting scientific development. It is of utmost importance to the contraceptive evaluation program of the Institute.

Proposed Future Course: Since all the original objectives of this project have been achieved, work during the forthcoming contract period will shift towards the new objective developed during the last contract period. Effects of OCA induced alterations in B₆ status and/or tryptophan metabolism on carbohydrate metabolism. The mechanism of this metabolic alterations giving rise to the diabetogenic effects of oral contraceptives is still unclear and will be characterized.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Interactions of Contraceptive Steroids with Metabolic Functions of Vitamin B₆
Contractor: University of Pennsylvania School of Medicine
Money Allocated: \$126,000 (FY 1973 Funds)
\$136,000 (FY 1974 Funds)

Objectives: The purpose of the proposed study is to perform a statistically valid analysis of clinical and chemical disturbance of B₆ metabolism in women on oral contraceptive pills and in pregnant women. This should lead to the establishment of necessity of B₆ supplementation in the above two conditions and to the desirable dose level of supplementary pyridoxine. Accompanying clinical conditions, like depression, edema, rheumatoid manifestations, will receive special attention.

Major Findings: A sensitive procedure (tyrosine decarboxylase assay) has been developed for measuring serum pyridoxal phosphate levels. Data thus far obtained show that plasma pyridoxal phosphate levels are reduced with OCA use and that this effect may be reversed with pyridoxine supplementation. Erythrocyte glutamic GOT values correlated significantly with plasma pyridoxal phosphate levels. The studies are continuing to provide information on more OCA users and on possible long-term OCA effects. Serum pyridoxal phosphate levels of 5 ng/ml and below are considered vitamin B₆ deficient, while normal values are 14 ng/ml and above. Dr. Gyorgy has also analyzed serum pyridoxal phosphate levels and erythrocyte GOT activities in samples of OCA users and non-users obtained from the study of Dr. Prasad. Depression in erythrocyte GOT activities were also observed in OCA users.

Significance to Biomedical Research and Program of the Institute: The proposed project may provide information on mechanism and consequences of altered vitamin B₆ metabolism in contraceptive users. In all published studies on abnormalities of vitamin B₆ status resulting from use of contraceptive steroids and pregnancy, primary or exclusive reliance was placed on the tryptophan load test or the determination of blood transaminases. This was due to lack of precise, reliable and reproducible analytical procedures for estimating pyridoxal phosphate (PLP) directly. In 1970 Chabner and Livingston described a method for the estimation of plasma PLP based on the measurement of ¹⁴CO₂ evolved from the decarboxylation of l-tyrosine-1-¹⁴C by tyrosine decarboxylase. The amount of ¹⁴CO₂ produced is proportional to the amount of PLP added to an excess of tyrosine apodecarboxylase. A modification of this procedure to be employed in the proposed project may offer new insight in the relationships between contraceptive use, primary manifestations of altered B₆ status and its secondary consequences, such as an abnormal tryptophan metabolism.

The dosage of pyridoxine supplementation necessary to reverse abnormal tryptophan load tests is a pharmacological one and greatly above what is known as the requirement for this vitamin. Such a high dosage may be necessary to

correct all abnormalities in tryptophan metabolism in all contraceptive users. This provides for simplicity of experimental design but limits reliable estimation and interpretation of altered dietary requirements of this nutrient. The experimental design underlying the project is considered outstanding both in regard to its epidemiological-clinical components and the nutritional-biochemical methodology.

Proposed Future Course: Quantitative correlations between all the various indices of vitamin B₆ status in OCA users have not been established. Work during the forthcoming contract period will focus towards establishing and characterizing these correlations: 1) pyridoxal phosphate levels by tyrosine decarboxylase assay, 2) alterations in tryptophan metabolism as detected by tryptophan load tests; 3) transaminase activities (GPT, GOT) and their PLP stimulation. Clinical manifestations and consequences of established abnormalities and correlations of abnormalities are to be explored.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Relationship of Effect of Oral Contraceptives on Blood Lipids and Nutritional Status
Contractor: St. Louis University, School of Medicine, St. Louis, Mo.
Money Allocated: \$58,500 (FY 1973 Funds)
\$65,000 (FY 1974 Funds)

Objectives: It has been postulated that increasing the level of estrogen stimulates the production of corticoisteroid which is responsible for the increase in the proteins that transport the lipids and other nutrients involved. A similar mechanism is considered to be responsible for the changes obtained in tryptophan metabolism. An objective of this present research is to attempt to determine whether the alterations in tryptophan metabolism can be correlated with the increases in blood lipids. Concurrently, evaluations of blood vitamin E, vitamin A, copper and ceruloplasmin are being made. These latter compounds may also increase in plasma concentrations as a consequence of increased protein carrier production. Serum ascorbic acid is also being determined because of reports that ascorbic acid blood levels are decreased after estrogen administration.

Major Findings: The increase in blood lipids observed in OCA users correlates with the increase in post-tryptophan xanthurenic acid excretion for most of the subjects tested to date. For some unexpected and unexplained reason, the best correlation is with the serum phospholipids. The vitamin E and vitamin A levels correlated with the lipids in the serum as expected, indicating that such changes in fat-soluble vitamins are a function of the increase in the protein carriers and are not necessarily related to the nutritional state of the tissues.

The single determination which best distinguishes those with and without OCA is copper in the serum. Some of the serum coppers in subjects taking OCA are as high or higher than one obtains in patients in the third trimester of pregnancy.

Significance to Biomedical Research and the Program of the Institute: The project may provide better knowledge of the long-term medical effects of oral contraceptives. Consequently, it is relevant to this program. Although the plans were based on many hypotheses, they were considered scientifically sound and targeted towards providing relevant information.

Proposed Future Course: Further work will be directed towards assessing the metabolic and clinical consequences of the biochemical changes observed in the previous contract period. In the basic science component of the project the metabolic significance of the abnormal tryptophan load test in OCA users will be characterized. Modification of this test is being investigated in order to increase the value of information derived from it and make it more applicable to screening of larger populations of OCA users.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: The Effects of Contraceptive Steroids on Trace Elements
Contractor: University of California at Berkeley
Money Allocated: \$87,000 (FY 1974 Funds)

Objectives: Definitive information pertaining to the effect of oral contraceptive steroids on trace elements is missing. While many observations have been made pertaining to the influence of OCA on iron, copper and to a lesser extent, zinc metabolism for the most part these observations are directed towards determination of blood levels without regard to trace mineral balances. The objectives of the project are to investigate the effects of contraceptive drugs on trace elements, balance of iron, copper and zinc in women kept on controlled diets in a metabolic ward.

Major Findings: Due to numerous technical and procedural difficulties inherent in trace element balance studies, the project has not yet yielded significant results of major importance. Difficulties and delays encountered were in establishing appropriate diets and conditions for human trace element studies and in developing satisfactory trace element analytical procedures. Both chromate and polyethylene oxide will be used as fecal markers in the mineral balance studies. Diets containing the FAO recommend protein allowance for adult women (0.52 gm/kg body weight) proved inadequate and resulted in negative nitrogen balance. An intake of 0.60 gm/kg body weight was satisfactory.

Significance to Biomedical Research and Program of the Institute: The proposed study of iron, copper, and zinc is justified and desirable. These three trace elements are known to be essential. For two of them (iron and zinc) we suspect or know that nutritional problems of suboptimal intake exist in some population groups, and all three are known to be in some way influenced by contraceptive metabolic changes, but they can be expected to furnish much needed information on the minimum daily requirement for these trace elements under normal conditions and with contraceptive medication. Only through carefully controlled metabolic studies within a metabolic ward can one accurately define the influence of contraceptive steroids upon mineral nutrition. The investigators possess one of the few unique capabilities in this country to conduct such detailed studies and the staff with the knowledge to provide adequate supervision for such a demanding project.

Proposed Future Course: Efforts will initially be concentrated towards overcoming the remaining technical and procedural difficulties and obstacles which have impeded progress. After this is accomplished a rigidly controlled metabolic balance study will be performed. Subjects are 6 young female graduate students, who will be maintained under rigidly controlled conditions in a metabolic facility for 17 weeks. They will be fed a constant formula type diet containing all known essential nutrients. Observations to be made will be analysis for all losses including urinary, fecal, skin and menstrual losses;

intakes will be determined by analyses of the diet. The subjects will receive in a randomized fashion two months of no steroidal agent, one month of a sequential type oral contraceptive and one month of a combined estrogen-progesterone agent. In addition to studies of trace elements, they will also further evaluate the effects of contraceptive steroids on calcium, magnesium and nitrogen balances. These balances will be particularly observed in regard to cyclic variations. Blood samples will be obtained and analyzed three times per menstrual cycle. Basal metabolic rates and requirements to perform a fixed amount of work will be determined throughout the cycle. Body weight variations will be observed. The trace mineral balance studies are primarily concerned with iron, copper, and zinc, employing conventional methods of analysis (absorption spectrometry for zinc and copper and calorimetric analysis for iron).

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Investigation of a Possible Interaction of Contraceptive Steroids with Vitamin E and Polyunsaturated Fatty Acids
Contractor: University of California - Los Angeles, California
Money Allocated: \$58,500 (FY 1973 Funds)
\$65,000 (FY 1974 Funds)

Objectives: It has been observed that in the rat the effects of administering oral contraceptive compounds resemble those resulting from vitamin-E deficiency. These include interference with reproduction, changes in lipoprotein distribution, and a decrease in polyunsaturated fatty acids in various tissues. The possibility exists that these anovulatory drugs enhance the requirement for vitamin E. Therefore, this investigation is designed to study the possible interrelationships between oral contraceptives and vitamin E. It is possible that oral contraceptive treatment results in situations where increased vitamin E and possibly increased polyunsaturated fatty acids are required for some essential physiological processes.

Major Findings: Rats were fed Enovid-E in conjunction with controlled intakes of vitamin E. The OCA appeared to lower plasma vitamin E levels and liver vitamin A levels. The plasma of α/β lipoprotein ratio changed with OCA administration (1.63 vs. 1.44 with OCA). The change appeared to be due an increase in serum β -lipoproteins. The effects observed with the rat were also observed in human subjects on OCA. Results must still be considered preliminary.

Significance to Biomedical Research and Program of the Institute: The project addresses itself to the problem whether oral contraceptives affect the vitamin E status of young women. This is relevant to the objectives of this program.

Proposed Future Course: Results and findings obtained so far shall be consolidated, extended and confirmed in a larger number of subjects. The biochemical and clinical significance and consequences of the observed alterations shall be ascertained. A comparative survey of vitamin E status of young women given oral contraceptive drugs for various lengths of time, with untreated young women serving as controls, will be done. This will involve determinations of plasma tocopherol levels, plasma cholesterol and alpha/beta lipoprotein ratios

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Biochemical Mechanisms of Contraceptive Steroid-Induced Abnormalities in Absorption, Metabolism and Function of Vitamin K
Contractor: University of Nebraska College of Medicine
Money Allocated: \$45,000 (FY 1974 Funds)

Objectives: The purpose of this project is to elucidate the mechanism of estrogen-induced changes in the requirement for vitamin K-dependent clotting proteins, and the decrease in sensitivity to oral anticoagulants. Specific objectives are to: (1) Determine the effect of estrogen and androgen on the quantitative response to deficient rats to vitamin K, (2) Characterize the effect of estrogen on oral anticoagulation in the rat, and (3) Ascertain the localization of vitamin K and the activity of vitamin K metabolizing enzymes in the liver of estrogen-treated animals.

Major Findings: Within the initial period of this project satisfactory progress has been reported in all lines of investigation directed towards the above objectives. The progress achieved suggests that all objectives, as specified above, will be achieved in the second contract period. Major findings have not yet been crystallized. Preliminary results have focused on an important tentative hypothesis, that a microsomal polypeptide precursor of prothrombin could be altered by estrogens and account for its coagulatory effect, and that of related synthetic compounds. Additional progress during the end contract period has led to the following findings and conclusions: (a) the higher levels of plasma prothrombin in female rats results from faster formation of the clotting protein; (b) the reduced requirement for vitamin K in female rats results from a decrease in the effective concentration of vitamin K in the liver, a phenomenon which requires time and does not appear to include direct substitution of vitamin K by estradiol; and (c) estrogen stimulates epoxide reductase. Thus, estrogen appears to permit vitamin K to act more effectively and rapidly.

Significance to Biomedical Research and Program of the Institute: The most well documented serious non-contraceptive effects of the anovulatory steroids is the higher incidence of thromboembolic disease, resulting from such treatment. Vitamin K has been implicated in this syndrome more than any other nutrient. In general, there appears to be a positive interaction between vitamin K and the steroids which may be the result of "K"-like activity from the steroid or the steroid increasing the efficiency of K utilization. This study proposes to investigate this interaction by examining the effect of the steroid on K absorption, metabolism and utilization.

Proposed Course: The scope of the project involves three lines of investigation. In the first part of the study, the requirement for vitamin K under various regimes of androgen and estrogen administration will be determined. In these studies, K-deficient rats with and without steroids will be given various levels of K in the diet. Blood samples will be assayed for K and for clotting factors such as prothrombin. In the second line of investigation, the effect of androgen and estrogen on retention and distribution of vitamin K in subcellular fractions of liver will be assessed. The influence of steroids on the enzymes responsible for the interconversion of vitamin K and its 2,3 epoxide will be characterized. A third line of work will attempt to characterize the mechanism of relative resistance to oral anticoagulants observed in pregnancy and under the influence of oral contraceptives. On the basis of his previous studies the applicant suggests that genetic resistance to anticoagulants may be due to an alteration in a vitamin K metabolizing system which is normally sensitive to warfarin and responsible for the anticoagulant effect. The experimental approach used to examine the warfarin resistant trait will be applied to the relative resistance to warfarin elicited in pregnancy and the estrogen-treated animal. The contractor plans to study further the mechanism of vitamin K function and the differences in vitamin K requirements that appear to exist between male and female rats.

NICHD ANNUAL REPORT
July 1, 1973 through June 20, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Effect of Oral Contraceptive Steroids on Calcium, Phosphorus, Magnesium and Zinc Metabolism, with Emphasis on Bone Mineralization
Contractor: Veterans Administration Hospital, Washington, D.C.
Money Allocated: \$39,276 (FY 1974 Funds)

Objectives: This proposal is designed to study the effect of steroidal contraceptive agents on mineral metabolism. The influence of a progestin, an estrogen, and a combination of these hormones on calcium, phosphorus, magnesium and zinc metabolism will be investigated. Particular attention will be focused on bone metabolism.

Major Findings: Major efforts were necessary to overcome technical and operational problems. The investigative component of the project had been delayed but has now entered into an orderly and systematic productive phase. The studies thus far conducted have demonstrated that OCA depressed serum zinc levels (96 ug/100 ml serum vs 69 ug/100 ml serum for OCA fed rats). The progestin component of OCA had less effect on depressing serum zinc levels than the estrogen component. Calcium, magnesium and phosphorus levels in serum do not appear to be affected to any marked degree by OCA ingestion.

Significance to Biomedical Research and Program of the Institute: The effect of anovulatory steroids on calcium, phosphorus, magnesium and zinc has not been established in a definitive manner. All of these mineral nutrients are important in the continuous process of bone formation and resorption. No known calcium balance studies have been carried out in human subjects receiving the oral contraceptives. There is conflicting evidence in the literature regarding the effect of anovulatory steroids on serum calcium, phosphorus, magnesium and zinc. Some, but not other investigators, have seen decreases in serum calcium, phosphorus, and magnesium during steroid ingestion; only the decreases of serum zinc appears well established. None of the available information allows a conclusion as to the effect of steroids on the nutrition requirement of these minerals. It can be expected that the proposed study will show whether or not the contraceptive steroids produce an increased requirement, as expressed by negative balance. Particular emphasis is to be placed on calcium which will be studied at three dietary levels. The duration of the experiment is enough to allow for adaptation of the animals to low or high calcium intakes, so that a valid interpretation can be made. It is important to assess the effect of the anovulatory agents on mineral metabolism since their prolonged use at a critical time (pre-pregnancy and pre-susceptibility to osteoporosis) may be detrimental to bone integrity.

Proposed Course:

The work required to complete the contract objectives includes the following:

1. Complete the analysis of all biological samples necessary to finish, evaluate and report the mineral balance study.
2. Determination of the fresh weight, fat free dry weight, ash weight, specific gravity, length and amount of organic material in the bone (femur).
3. Histological evaluation and cortical thickness of the femur.
4. Statistical computations and summarization of all data.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: The Interaction of B-Vitamins with Contraceptive Steroids in Female Rats
Contractor: School of Pharmacy, University of Georgia, Athens, Georgia
Money Allocated: \$26,300 (FY 1973 Funds)
\$35,000 (FY 1974 Funds)

Objectives: The purpose of this project is to investigate in the rat the effects of vitamin B complex deficiencies and hypervitaminosis states on the metabolic hydroxylation of selected steroids, and the effects of contraceptive steroids on the function and disposition of thiamin, riboflavin and pyridoxine. Deficiency and hypervitaminosis B states have been shown to alter the activity of certain liver enzymes. Several of the B complex vitamins are constituents of liver enzyme systems involved in the metabolic disposition of steroids.

Major Findings: Thiamin and riboflavin deficiency states increase the rates of metabolism of certain drugs by hepatic microsomes. These states cannot be considered as inducers of drug metabolizing enzymes in the same sense as enzyme induction by drugs such as Phenobarbital, because no elevation of liver weight/body weight ratio, microsomal protein content, cytochrome c reductase activity or cytochrome P-450 is observed. Evidence from the investigator's laboratory suggest that thiamin deficiency states may alter the character of the cytochrome P-450 which in turn may be responsible for these changes. Further results obtained indicate that metabolism of mestranol was 2.3, 2.2 and 1.5 x higher in rats fed thiamin, riboflavin or pyridoxine deficient diets than in rats fed high levels of these vitamins. It appears that these changes are accompanied by changes in the activity of NADPH cytochrome c reductase and/or cytochrome P-450 content. The effect of the diet appears to be most pronounced on the membranes of the endoplasmic reticulum, although riboflavin and pyridoxine may affect enzymes of the soluble cytoplasm which in turn modify microsomal drug metabolizing enzymes. The administration of mestranol and norethindrone induce changes in the activity of drug metabolizing enzymes in a manner which appears governed by the diet; however, the mechanism of this interaction is yet to be elucidated in the future course of this project.

Significance to Biomedical Research and the Program of the Institute: Many reports have implicated contraceptive steroid preparations in the modification of B vitamin needs, but no evidence of the mechanism of this action has as yet been obtained. More importantly perhaps, the alternate problem has not been investigated, i.e., the effect of vitamin deficiency on steroid metabolism. It is apparent that the proper action of a drug such as these steroids depends on maintaining the balance between uptake and catabolism. Anything that modifies this pattern can result in either decreased efficiency or increasing deleterious side effects. The B vitamins are known to have a role in the oxidation and hydroxylation of steroids as well as maintaining the general metabolic integrity of the cell. This project is designed to explore this subject.

It thus could potentially provide fundamental information concerning the safety of the use of oral contraceptives.

Proposed Course: This is defined by the considerable progress made in the past contract period. It has generated many interesting although poorly understood observations. Additional experimentation will be required to clarify the OCA effects observed on drug metabolizing enzymes, particularly with respect to cytochrome P-450. Localization of the subcellular site affected by dietary vitamin intake should be completed; also the study designed to determine the effects of B vitamins on the subcellular constituents of hepatic drug metabolizing enzymes. An attempt will also be made to elucidate the mechanism by which contraceptive steroids alter mixed function oxidases of hepatic microsomes in the presence of varying nutritional status.

Since multi-vitamin hypervitaminosis or deficiency states are more likely to occur in human populations, experiments designed to define the effects of these conditions on the drug hydroxylase system responsible for metabolizing oral contraceptives and other drugs will be undertaken. Drug hydroxylase activity, cytochrome P-450 content and NADPH cytochrome c reductase activity will be used to indicate alterations in this complex system.

NICHD ANNUAL REPORT
July 1, 1973 through June 22, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title : The Effects of Oral Contraceptive Steroid Hormones
on Folic Acid Metabolism
Contractor : University of Alabama/School of Medicine/The Nutrition
Program (P.I.=C.E. Butterworth, Jr., M.D., Professor
of Medicine and Pediatrics, Director, The Nutrition
Program)
Money Allocated: To be negotiated
Money Requested: To be negotiated - approximately \$75,000 (for first year)

- Objectives: 1) Perform a baseline study to establish the level of serum folate as a function of normal menstrual cycle and OCA "pill cycle" by means of two bioassays: Lactobacillus casei and Streptococcus fecalis. In this baseline investigation, the hypothesis shall be tested that during the estrogenic (follicular) phase, and under the influence of exogenous steroids there will be an increase in non-methylated folate species in serum, in contrast or in comparison to total serum folates.
- 2) Determination of frequency (prevalence) of abnormal folate levels in OCA users and non-users (controls).
- 3) Determination of plasma conjugase activity (PPGH=pteroyl polyglutamyl hydrolase).
- 4) Characterization of intestinal absorption and urinary excretion of folates in OCA-users and non-users (controls).

Major Findings: None - The project has not yet become operational.

Significance to Biomedical Research and Program of Institute: This research program offers, for the first time, realistic hopes, expectations and concrete scientific leads for a common, unifying pathogenesis of diverse apparently unrelated adverse medical effects of oral contraceptives. Cervical dysplasia, mental and mood changes, thrombosis, hypertension and hyperpigmentation may conceivably be sequels of specific and possibly different kinds of folate deficiency. If this can be demonstrated in the proposed program, all these adverse effects could be prevented and/or reversed by administration of pteroylglutamic acid (monoglutamic folic acid).

Proposed Course: In order to achieve objective #1, the level or pool of non-methylated folates shall be determined by the Streptococcus Fecalis Assay, since this organism does not reflect or indicate the presence of methylated folates, specifically of N-5 methyltetrahydrofolate. The level and pool size of total folates (both methylated and non-methylated forms of folate) shall be determined by the Lactobacillus Casei Assay, which reflects and indicates the presence of both methylated and non-methylated folate species. Measurements are to be carried out every other day during the course of one or several

menstrual or pill cycles, on a small number of approximately 10 subjects.

In order to achieve objective #2; the same microbiological assays described above, will be employed. Serum samples are to be collected at a constant, fixed time period of the menstrual or pill cycle for all subjects (e.g., between 10th and 20th day of cycle). The sample size should consist of approximately 100 subjects in each of the two groups. Rationale for selection of sample size: (1) 10% of subjects receiving diphenylhydantoin develop low levels of serum folates; (2) approximately 20% of OCA users developed megaloblastic changes in cervical smear, which were reversed by administration of 10 mg pteroylglutamic acid (PGA=Folic acid monoglutamate=monoglutamic folic acid).

The investigative course for objective #3, follows from the following rationales: The functional folate co-enzyme is probably polyglutamate in nature. The predominant molecular species of the folate in plasma and urine is monoglutamate. The ratio between the two molecular forms or species of folate is largely determined by the enzyme "Plasma Conjugase." Increased activity of this enzyme could lead to urinary losses and folate deficiency. In order to obtain a detailed picture of the nutritional folate status of OCA users, the profile of criteria ought to include determination of PPGH in addition to the two folate levels (as specified above - b). The sample size, composition and epidemiologic protocol for determination of conjugase activity ought to be of the same magnitude as for the determination of folate levels since similar epidemiologic rationale are applicable.

The investigative course for objective #4 follows from the following rationales: The first published report on "Folate Deficiency in OCA users (Streiff, R.R.: JAMA 214: 105, 1970), included the observation that only those OCA users develop folate deficiency in whom OCA's interfered with the absorption of naturally occurring polyglutamic folic acid in their food. It was therefore implied that the pathogenesis of folate deficiency required at least two or three essential etiologic or causal factors: (1) a genetic factor, determining the susceptibility of a fraction of OCA users to the effect of OCA's on the intestinal absorption of dietary, e.g., polyglutamic folate; (2) the exposure to OCA's and, (3) the chemical species of dietary folate had to be polyglutamicfolic acid. Dietary administration of PGA (pteroylglutamic acid = folic acid monoglutamate - monoglutamic folic acid) therefore corrected folate deficiency in spite of the continued operational presence of the other two etiologic factors (1+2). The work and conclusions of Streiff have not been confirmed; it has been disputed by contradictory arguments of even more uncertain miscellaneous results. (References available). A rigorous, unequivocal test for the absorption of pteroyl polyglutamate requires the utilization of a well-defined synthetic pteroyl polyglutamate of known and reproducible structure and composition. Dr. Butterworth has at his disposal a limited amount of synthetic pteroyl polyglutamates of each chain length (mono-, di-, tri-, tetra-, penta-, hexa-, and hepta-), along with the capabilities of synthesizing them by the solid-phase method. He also has completed and published definitive study on intestinal absorption of pteroyl glutamic acid (PGA=monoglutamic folic acid) and folic acid.

NICHD ANNUAL REPORT
June, 1974 through next F.Y.
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title : Prospective Study of the Effects of Contraceptive Agents, Related Methods, and Procedures on the Frequency of Birth Defects

Contractor : Kaiser-Permanente Medical Center, Walnut Creek, California (P.I.=Savitri Ramcharan, M.D., Ph.D.)

Money Allocated : Under Negotiation to reduce money requested, if possible

Money Requested : \$416,349 (For first year).

Objectives: The objective is to evaluate the effects of contraceptive agents, related methods and procedures on the health of the fetus and offspring. The projected time for completion of the project is 36 months. The objectives are to be achieved by a prospective cohort analysis starting with pregnant women and following the outcome of their pregnancies.

Major Findings: None - The project has not yet become operational.

Significance to Biomedical Research and Program of the Institute: Congenital defects resulting from contraceptive failure or presently available methods of contraception may represent a major adverse effect and poses a public health problem. The proposed project will provide quantitative yardsticks to measure and access this problem. It is therefore highly relevant.

Proposed Course: A prospective study is proposed to obtain information about contraceptive use from female members of the Kaiser Foundation Medical Care Program receiving prenatal care at Kaiser-Permanente medical clinics, and to relate this data to the information about pregnancy outcomes and neonatal and infant abnormalities obtained from the inpatient and outpatient maternal and infant medical records.

The data base is to consist of the records and examinations of 31,000 deliveries, infants and their mothers. Maternal data will comprise type of contraceptive used during the year preceding conception, maternal health conditions, history of previous pregnancies and outcomes as well as a drug history. This historical information will then be related to the pregnancy outcomes, since both prenatal and ante-natal care of mother and infant are carried out within the Kaiser system.

Intake of subjects into the study will last 18 months and yield a total of 31,000 live births. It is anticipated these total samples of mothers breaks down into three groups: a) 12,500 will have a history of oral contraception; b) 12,500 will have a history of other methods of contraception; c) 6,000 will have no history of contraception.

NICHD ANNUAL REPORT
June, 1974 through next F.Y.
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title : Contraceptive Agents and Birth Defects
Contractor : Harvard School of Public Health, Boston, Massachusetts
(P.I. Kenneth J. Rothman, Ph.D.)
Money Allocated : Under Negotiation to reduce money requested
Money Requested : \$87,130 (for first year)

Objectives: The objectives of this project are to determine the effects of oral contraceptives and hormonal pregnancy tests, administered to mothers, on the risk of developing congenital malformations and other characteristics of the offspring.

Major Findings: None - The project has not yet become operational.

Significance to Biomedical Research and Program of the Institute: Congenital defects resulting from contraceptive failure or presently available methods of contraception may represent a major adverse effect and poses a public health problem. The proposed project will provide quantitative yardsticks to measure and access this problem. It is therefore highly relevant.

Proposed Course: The objectives of this contract shall be achieved by implementation of two epidemiological studies:

1. A retrospective case-control study on 500 cases of congenital malformations of the heart.
 - a) Cases shall be identified, diagnosed and obtained from the New England Regional Infant Cardiac Program (NERICP). Efforts shall be made and procedures developed to check and verify the diagnosis established by NERICP.
 - b) 1000 controls of normal infants shall be selected from a random sample of all births within the state of Massachusetts during the intake period of cases.
 - c) Contraceptive histories and related drug exposures shall be collected by self-administered, mailed questionnaires. Additional and special efforts shall be made and procedures developed to check and verify these data.
 - d) Risk ratios for particular malformations among selected exposed groups, relative to those unexposed shall be determined by standard epidemiologic techniques.
 - e) The intake period for the collection of cases and controls shall extend for 30 months.

f) Coding, editing and analysis of edited data shall overlap with the period of data collection and shall be completed within 36 months after the beginning of the study.

2. A prospective study to evaluate the effects of oral contraceptives administered to mothers on congenital malformation and health status of their offspring.

- a) From an established cohort of 66,000 women currently followed for cancer occurrence approximately 20,000 women with a history of oral contraceptive use shall be identified.
- b) The selected cohort shall include the following sub-cohorts of women who (1) use oral contraceptives until they became pregnant, (2) became pregnant within 3 months of discontinuing oral contraceptives, (3) became pregnant within an unknown interval after discontinuation of oral contraceptives, (4) had used oral contraceptives, but not used them within 12 months prior to pregnancy, (5) never used oral contraceptives prior to pregnancy; e.g., controls.
- c) The outcomes of the pregnancies of all women in the five subcohorts shall be determined by abstracting clinical records of deliveries and infants. The data shall include, sex, congenital defects and anomalies, birth weight and other biological parameters relevant to the health status of the infant.



NICHD ANNUAL REPORT
June, 1974 through next F.Y.
Fertility Regulating Methods Evaluation Branch
Contract and Collaboration Research

Contract Title : Methods of Contraception, Pregnancy Tests, and Congenital Malformations: An Epidemiological Study
Contractor : Yale University School of Medicine, New Haven, Connecticut (P.I. Colin White, B.Sc., M.Sc., M.B., Professor of Public Health)
Money Allocated : Under Negotiation to reduce money requested.
Money Requested : \$122,427 (for first year)

Objectives: The objectives of this project are to determine the effects of oral contraceptives, other methods of contraception and hormonal pregnancy tests, administered to mothers, on the risk of congenital malformations in their offspring.

Major Findings: None - The project has not yet become operational.

Significance to Biomedical Research and Program of the Institute: Congenital defects resulting from contraceptive failure or presently available methods of contraception may represent a major adverse effect and poses a public health problem. The proposed project will provide quantitative yardsticks to measure and access this problem. It is therefore highly relevant.

Proposed Course: The objectives of this contract shall be achieved by operational implementation of a retrospective case control study. The population to be investigated shall consist of all infants born with congenital defects and abnormalities in five large Connecticut hospitals, or referred to these hospitals, in a period of two years.

- a. Cases shall be identified, diagnosed and obtained from these hospitals.
- b. Additional efforts shall be made to check and verify accuracy of diagnosis and completeness of case ascertainment.
- c. From an estimated 34,000 births over a two year period, efforts will be made to ascertain approximately 575 cases with congenital heart disease and approximately 800 other defects (neural tube defects, pyloric stenosis, hypospadias, undescended testicles, Down's syndrome, cleft palate and cleft lip, club foot, congenital dislocation of the hip, and others).
- d. Controls shall be obtained from a random sample of normal births each day of case ascertainment. The ratio of number of controls to number of cases shall be 3:1.
- e. Information and data on contraceptive use as well as other important variables will be obtained by a standardized questionnaire administered by trained interviewers to the mothers of cases and controls. This shall be supplemented, and verified by checks with the physician and his records.

NICHD ANNUAL REPORT
June, 1974 through next F.Y.
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: : The Effect of Oral and Other Contraceptives on
Congenital Abnormalities and Outcome of Pregnancy
Contractor : Hebrew University-Hadassah Medical School, Jerusalem,
Israel, (P.I. Dr. Susan Hailup, Lecturer in Epidemiology)
Money Allocated : Under Negotiation to reduce money requested, if possible.
Money Requested : \$33,429 (for first year)

Objectives: The objectives of this project are to 1) measure the effects of contraceptives used prior to, and at the time of conception, on outcome of pregnancies, congenital defects and chromosomal aberrations. 2) measure pregnancy-outcomes in relation to age of ova at the time of conception.

Major Findings: None - The project has not yet become operational.

Significance to Biomedical Research and Program of the Institute: Congenital defects resulting from contraceptive failure or presently available methods of contraception may represent a major adverse effect and poses a public health problem. The proposed project will provide quantitative yardsticks to measure and access this problem. It is therefore highly relevant.

Proposed Course: The objectives of this contract shall be achieved by the following studies, methods, procedures and analyses:

- 1) Conduct a retrospective cohort study of 14,000 births occurring during the first two years of this three-year contract project.
- 2) Obtain all relevant information on 15-20,000 previous births of the same women since 1964, by record-linking with the Jerusalem Perinatal Study.
- 3) Time schedule, methods, procedures, protocol and analysis, shall include:
 - a) All parturient women will be interviewed within 1-2 days of giving birth. They will be questioned about contraceptive behavior before and at the time of conception, their intention to conceive, and the length of time trying. For those who have ever taken the pill, a detailed history of this will be recorded. Specific information will be sought on the use of hormone pregnancy tests and preparations intended to induce or prevent abortion, and on smoking, religious observance, gynecologic morbidity in early pregnancy, as well as menstrual history.
 - b) Quantitative ascertainment of pregnancy outcome - this is to include incidence of fetal and neonatal deaths, complications of pregnancy, methods of delivery, health status of the offspring (birthweight, congenital abnormalities, chromosomal aberrations, fetal distress or asphyxia).

c) Data evaluation by multiple regression analysis to determine the relationships between demographic and health variables to contraceptive behavior and the relationship of contraception to pregnancy outcomes. Possible interactions between oral contraceptives and other health variables such as smoking are also to be explored, using regression techniques and traditional methods of analyses of specific rates.

NICHD ANNUAL REPORT
June, 1974 through next F.Y.
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title : Effects of Contraceptive Agents, Related Methods and Procedures and Frequency of Birth Defects
Contractor : University of Colorado Medical Center, Denver, Colorado
(P.I. James J. Nora, M.D. Associate Professor of Pediatrics)
Money Allocated : Under Negotiation to reduce money requested, if possible.
Money Requested : \$73,540 (for first year)

Objectives: The objectives of this project are to determine whether or not hormonal contraceptives and other contraceptive measures constitute a fetal risk, produce fetal loss, congenital malformations and chromosomal aberrations.

Major Findings: None - The project has not yet become operational.

Significance to Biomedical Research and Program of the Institute: Congenital defects resulting from contraceptive failure or presently available methods of contraception may represent a major adverse effect and poses a public health problem. The proposed project will provide quantitative yardsticks to measure and access this problem. It is therefore highly relevant.

Proposed Course: The objectives of this contract shall be achieved by the following epidemiologic studies:

1. A case-control studies on 500 infants with malformations of the heart, 150-200 infants with chromosomal anomalies and 500 other malformations. Two matched controls will be selected for each case. Maternal contraceptive history will be obtained by interview.
2. A retrospective and prospective cohort study. Each shall contain 500 pregnancies which had been exposed to contraception. Ascertainment of cases and controls in the retrospective cohort study shall be initiated by reviewing of charts compiled over the past five years. The population base for the prospective cohort study shall consist of 500 future pregnancies which have been exposed to contraception. Starting point will be their ascertainment and identification in the participating clinics. Data on the infants in both cohort studies will be obtained by direct examinations. The results of these will be checked and verified by the investigators and their consultants.

NICHD ANNUAL REPORT
June, 1974 through next F.Y.
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: : Evaluation of the Effects of Contraceptive Agents,
Related Methods and Procedures and Frequency of
Birth Defects

Contractor : New York State Department of Health, Health Research, Inc.
Albany, New York (P.I. Dwight T. Janerich, D.D.S., M.P.H.)

Money Allocated : Under Negotiation to reduce money requested, if possible.

Money Requested : \$58,895 (for first year)

Objectives: The primary, immediate objective of this project is to detect any association between contraceptive practices and subsequent birth defects. In the event that a positive association is found between pill failure and any or all congenital malformation types, the Ob/Gyn Department of the Albany Medical College will develop a program for complete clinical endocrine evaluation of mothers of congenitally defective children who experienced unexplained oral contraceptive failure.

Major Findings: None - The project has not yet become operational.

Significance to Biomedical Research and Program of the Institute: Congenital defects resulting from contraceptive failure or presently available methods of contraception may represent a major adverse effect and poses a public health problem. The proposed project will provide quantitative yardsticks to measure and access this problem. It is therefore highly relevant.

Proposed Course: The proposed project comprises four epidemiological case-control studies of: limb reduction deformities (100 pairs), central nervous system malformations (200 pairs), mongolism (100 pairs), and multiple malformations including cardiovascular malformations (100 pairs). The types have been selected on the basis of suggested associations in the literature and case availability. Each case will be matched to a control on the basis of date of birth, county in which the birth occurred and maternal age. Matching on maternal age will provide an indirect match on parity, residual differences will be compensated for by stratification in the analysis of the data.

The data will be analyzed to detect significant associations between the use of contraceptive practice or failure of a contraceptive procedure and the frequency of birth defects. Possible associations between the use of hormone pregnancy tests and subsequent birth defects will also be explored. If any association is found between a contraceptive practice and the occurrence of a birth defect, it will be used to determine the nature of the association, whether it is due to teratogenesis or mutagenesis of the contraceptive measure which would be a primary relationship, or some secondary relationship.

The operational and data base of the currently proposed project is the New York Birth Defect Institute's Surveillance Program to ascertain cases, and

matched with controls at comparable birthdate, birthplace and maternal age. Maternal information about contraceptive usage, other medications, diet, etc., is obtained by interviews.

NICHD ANNUAL REPORT
June 1, 1974 through next F.Y.
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Level of Risk of Female and Male Offspring of Mothers
Exposed to Ingestion of Diethylstilbestrol During Pregnancy
Contractor: University of Chicago, Dept. of Obstetrics and Gynecology,
Chicago, Illinois (P.I. Marluce Bibbo, M.D., Assistant Prof.)
Money Allocated: Under Negotiation to Reduce Money Requested, if Possible
Money Requested: \$78,964 (For First Year)

Objectives: The objective of this project is to determine the level of risk for development of neoplastic lesions in female and male offspring of mothers exposed to ingestion of diethylstilbestrol during pregnancy.

Major Findings: None - the project has not yet become operational.

Significance to Biomedical Research and Program of the Institute: The proposal for this project was submitted in response to RFP-NICHD-ME-74-1: "Effects of Contraceptive Agents, Related Methods and Procedures on Frequency of Birth Defects." It was considered not directly responsive, but nevertheless closely related to the RFP and directly relevant to the mission of the FRMEB, CPR and NICHD. The important relevance was recently demonstrated and underlined since DES was approved as a post coital contraceptive. All pregnancies resulting from contraceptive failures are or might be potentially at the same risk as those when DES was administered for therapeutic purposes, 20 years ago. Further, the outcome and results of the project should provide quantitative criteria and yardsticks for initiation of public health measures; e.g., a program for identification and medical examination of all offspring from mothers who received DES during pregnancy.

Proposed Course: The objectives of this project are to be achieved by a "retrospective" cohort analysis. For this purpose 1,646 women who completed the course of treatment twenty years ago will be located, as well as their offspring. They were part of a double-blind placebo controlled investigation aimed at determining the value of DES in pregnancy by W. J. Dieckmann, M.E. Davis, L. M. Rynkiewicz, and R. E. Pottinger entitled "Does the Administration of DES during Pregnancy have Therapeutic Value?" (Am. J. Obst. and Gynec. 66:1062-1081, 1953). A total of 2,162 patients was entered in the study. The number actually completing the course of therapy was 840 in the stilbestrol group and 806 in the non-medicated control group. Graduated increasing dosages were administered beginning about the seventh week of gestation. The investigators concluded that stilbestrol did not reduce the incidence of abortion, prematurity, or postmaturity. It neither decreased the incidence of perinatal mortality, nor the frequency of the toxemias of pregnancy.

A follow-up study of the offspring of the patients who were involved in the study described above, which was conducted at the University of Chicago, provide a unique opportunity to answer important currently pressing and urgent questions of great public health significance. One of the most important is an estimate of the risk of those exposed, i.e., the risk of both female and

male offspring. Further, the possible effects of DES on the sexual development of these offspring and also for development of neoplasms in all gonadal ridge derivative especially the secondary sex organs need to be determined.

According to the investigative plan, answers to all these questions will be forthcoming during the course of a three year project period. Initially, a survey of existing records will be conducted to obtain data on fetal loss, abnormalities in fetus including congenital malformations, sex ratio in live birth and in all fetal deaths, and other characteristics of newborn of fetus. A follow-up study on the offspring will then be conducted in order to assess possible late effect of the DES on the sexual development and for possible presence of neoplasms in all gonadal ridge derivatives.

NICHD ANNUAL REPORT
June 1, 1974 through approximately 9 months
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Consequences of Contraceptive Failure in Terms of Birth Weight, Fetal Loss, Congenital Malformations, and Infant Mortality

Contractor: Child Health and Development Studies, School of Public Health, University of California, Berkeley, Oakland, California 94611

Money Allocated: Under Negotiation - to reduce money requested, if possible

Money Requested: \$21,970

Objectives: The objectives of this program initiative are to determine the consequence of contraceptive use at the time of conception in terms of fetal loss, low birth weight, infant mortality, and congenital malformations. In particular, comparisons shall be made of the outcomes of pregnancies where a) that method of contraception had been used in the interval since last pregnancy (or marriage) but deliberately discontinued, and b) no contraception had been practiced in the interval.

Major Findings: None- The project has not yet become operational.

Significance to Biomedical Research and Program of the Institute: Congenital defects resulting from contraceptive failure or presently available methods of contraception may represent a major adverse effect and poses a public health problem. The proposed project will provide quantitative yardsticks to measure and access this problem. It is therefore highly relevant.

Proposed Course: The general objectives of the new program initiative are similar to the ones of the other projects. The specific, essential and unique difference of objective in the new program initiative is to obtain valuable information within 6-9 months time. It is fortunate that the answers from the other approved projects will not be forthcoming until 3-5 years. This is unfortunate because the public health concern and need for answers is an urgent issue presently widely publicized. The reason for this long waiting period necessary for obtaining answers from projects presently approved and implemented lies in the absence of an established data base.

The project described in this section of the Annual Report, differs in one essential aspect from all other projects in the program. The data base is already established in:

Child Health and Development Studies
Division of Biostatistics
School of Public Health
University of California, Berkeley

Supported and funded by:

Grant No: HD00718

NIH

NIH

This permits an epidemiologic strategy of a "retrospective, prospective" analysis or perhaps more accurately a retrospective cohort analysis. This type of approach yields conclusions, which are perhaps less rigorously controlled than the ones from the other approved projects. They will, however, be forthcoming within nine months at a very low cost or cost-benefit ratio. This will at least fill the large time interval between current concern and conclusions expected from the other projects with meaningful, highly valuable information.

Scope and course of the project is determined by the available data base which retrospectively permits a cohort analysis of pregnancy outcomes, thus having the advantage of a longitudinal or prospective epidemiological design and strategy.

Such a unique data base is provided by the files of Child Health and Development Studies, University of California, Berkeley. The data in these files cover 15,886 pregnancies of interviewed women, terminating at Kaiser Hospital Oakland, California, between 1960 and early 1967. Women were referred to the Study by Kaiser Permanente Health Plan at their first contact with the OB-GYN clinic in connection with a possible pregnancy.

To be included in this project, women must have been interviewed and must have answered the questions regarding contraceptive use (the questions were not included in the interview until late 1960). In addition, the outcome of the pregnancy must be known, and the fact of pregnancy must have been verified; there are 14,326 pregnancies which meet these requirements.

Course and scope of project is limited to data analysis by standard epidemiologic techniques and procedures.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Vasectomy on Testes, Epididymis and Vas Deferens
Contractor: University of Virginia School of Medicine
Money Allocated: \$122,830 (FY 1974 Funds)

Objectives: This contract supports two related projects conducted by two investigators in the same department. One study is examining the function of smooth muscle in the vas deferens and changes which may occur as a result of vasectomy. The other utilizes micropuncture techniques to study intraluminal pressure, sperm morphology, and sodium-potassium levels in the seminiferous tubules and epididymis of normal and vasoligated animals. The effects of vasoligation on the permeability of the blood-testis barrier to tracer particles of various sizes are also under study.

Major Findings: Data on mechanical properties of the smooth muscle of the vas deferens have been obtained in vivo in normal and vasoligated rabbits. The data demonstrate that there are no spontaneous contractions in the vas of the normal rabbit but it contracts in response to stimuli. In contrast, the obstructed vas shows spontaneous contractions at 1 month and at 12 months after vasoligation. At 1 month after obstruction, the wall of the vas thickens, and at 2 months and later intervals, there is gross dilatation with thinning of the vasal wall.

Studies of the effects of vasoligation on intratubular pressure in the seminiferous tubules and epididymis in hamsters have demonstrated four-fold increases in pressure in the cauda epididymis while pressure in the seminiferous tubules and caput remains at normal levels. Further studies of these pressure changes and the effects of sperm granuloma formation and resealing are in progress in several animal species. Very preliminary data have been obtained on sodium and potassium content of intraluminal fluids of the seminiferous tubules, caput and cauda epididymis of the normal hamster. Sodium and potassium levels in seminiferous tubule fluid appear to be much higher than those in other physiologic fluids.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract are expected to provide new and much needed information on the physiological effects of vasectomy on organs of the male reproductive tract.

Proposed Course: It is expected that studies under this contract will be continued for several years.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: An Immunogenetic Analysis of Sperm Autoantigens in the Mouse
Contractor: Cornell University Medical College
Money Allocated: \$360,344 (FY 1973 funds for 3 years)

Objectives: The purpose of this contract is to study antigens on the surface of mouse sperm directed by genes at the T locus. The antigenicity and cross reactivities of products of wild type and mutant genes are being investigated in immunization experiments and will subsequently be studied after vasectomy.

Major Findings: Work under this contract has demonstrated that not only the sperm surface antigen specified by the gene T but also those specified by two recessive lethal alleles (t^0 and t^{w2}) can function as autoantigens. In each case antisera obtained from appropriate immunization experiments are specific for the t allele used for immunization. In alloimmunization experiments with sperm from mice carrying any one of four recessive alleles (t^0 , t^{w1} , t^{w5} , and t^{w32}), antisera have been produced which, after appropriate absorption, react only with sperm of the donor type; no cross reactivities within these four alleles have been detected. Cross reactivity between products of t^0 and t^{w2} alleles has been detected in cytotoxicity tests and will be checked further by absorption analyses. Syngeneic immunizations within the inbred normal mouse colony of this laboratory have been completed to produce sperm-specific antisera of high titer which, after appropriate absorptions, will be tested on sperm from mice of other inbred strains to determine whether there is polymorphism among wild type alleles at the T locus.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated last year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract are expected to lead to interesting and highly significant findings on the nature of gene products on the sperm surface which may act as autoantigens under such conditions as vasectomy.

Proposed Course: Substantial progress has been made in this project but complete definition of the surface antigens on mouse spermatozoa specified by genes at the T locus and determination of their autoantigenicity and role in an immune response to vasectomy are expected to require several additional years.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Immunologic and Morphologic Consequences of Vasectomy
Contractor: University of Missouri Medical Center
Money Allocated: \$203,653 FY 1973 funds for two years)

Objectives: The purpose of this program is to determine the morphological and functional changes subsequent to vasectomy, the role of the immune response in the development of these changes, and the possibility of protection against immunologically mediated injury by certain immunoglobulin classes. Studies are being conducted in histocompatible Strain 13 guinea pigs and in human patients. Humoral and cellular immune responses, fertility, and pathologic affects on reproductive and other tissues are being studied in immunized, vasectomized and vasoligated guinea pigs and the role of antibodies and cells in the development of pathology is being tested in passive transfer experiments. Studies in human subjects include determination of antisperm antibody levels, immunoglobulin classes, and delayed hypersensitivity before and at intervals after vasectomy.

Major Findings: Immunization of syngeneic Strain 13 guinea pigs with sperm with or without adjuvant leads to formation of antisperm antibodies as measured by sperm agglutination and indirect immunofluorescence at 2-29 weeks after immunization. There was no correlation between sperm agglutination titer and intensity of fluorescence. Tests for delayed hypersensitivity were negative at 16 and 29 weeks but the possibility remains that these animals passed through an earlier phase of delayed hypersensitivity. Animals immunized with sperm in complete Freund's adjuvant showed severe autoimmune aspermatogenesis, and lesser degrees of damage were seen in guinea pigs immunized with sperm in incomplete adjuvant or saline. Most unilaterally vasectomized or vasoligated Strain 13 guinea pigs demonstrate sperm agglutinating antibodies but no clear evidence of delayed hypersensitivity has been obtained. Preliminary data indicate diminished fertility in unilaterally vasectomized guinea pigs at 1 month and at 10 months after surgery and further experiments are in progress to determine whether this may be related to stress from the experimental procedures.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number supported by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies supported by this contract should contribute significantly to defining immunological mechanisms in experimental allergic aspermatogenesis and following vasectomy, and provide for correlation of data between the guinea pig model and vasectomized patients. Transfer experiments now being initiated should further clarify immunopathogenic mechanisms, and passive immunization may afford protection against immunologic consequences of vasectomy.

Proposed Course: It is expected that these and related studies will be continued for several years.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Vasectomy and Autoimmune Disease
Contractor: Memorial Hospital, Worcester, Massachusetts
Money Allocated: \$46,459 (FY 1973 Funds)

Objectives: The purpose of this contract is to support studies of the immune sequelae of autoimmune aspermatogenesis and vasectomy in mice. Immunoglobulin profiles and cellular immune responses are to be determined by a variety of techniques, evidence for autoimmune phenomena other than aspermatogenesis is to be sought, and histopathologic studies are to be conducted at autopsy with special attention to evidence for arthritis, endocrine and particularly adrenal tumors, and immune complex glomerulonephritis.

Major Findings: Screening tests have been performed seeking evidence of possible autoimmune responses in mice vasectomized for periods up to one year. No abnormalities attributable to vasectomy have been demonstrated in routine hematologic tests, serum immunoglobulin profiles (IgM, IgA, IgG₁ and IgG₂), antibodies to mouse immunoglobulin as detected by the Coombs technique, or serum levels of SGOT, leucine aminopeptidase, acid and alkaline phosphatase, and amylase. Some abnormalities in testicular histology may be attributable to the surgical procedure.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract could provide for comparison between the effects of experimentally-induced aspermatogenesis and vasectomy and thus should contribute important information on possible pathologic effects of vasectomy.

Proposed Course: Studies under this contract are expected to terminate at the end of the present fiscal year.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Immunologic Studies on Steroid-Producing Cells
Contractor: State University of New York at Buffalo
Money Allocated: \$120,000 (FY 1974 funds for two years)

Objectives: This contract supports investigations of steroid-producing cells of the testis and immunologic responses to these and other cells in vasectomy. Circulating antibodies to adrenal and testicular tissue are being sought in sera from vasectomized men and animals and animals immunized with testicular tissue and steroid-protein conjugates. Antigens of human and animal testicular tissue are being characterized by immunochemical and immunoenzyme methods.

Major Findings: Sera from 45 patients have been tested for antibodies to adrenal cortex, Leydig cells, theca interna and antinuclear antibodies by indirect immunofluorescence and immunoperoxidase techniques. No antibodies to testicular or ovarian steroid-producing cells have been demonstrated but antibodies to cells of the adrenal cortex were demonstrated in two patients 28 and 36 days after vasectomy and antinuclear antibodies were detected in three patients 28, 44, and 45 days after vasectomy. These antibodies were not present in serum samples obtained at the time of vasectomy. Antibodies to testicular antigens have been detected in 10 of 30 vasectomized rabbits and the time sequence of appearance and decline of these antibodies has been documented. Peak titers appear at about 220 to 340 days. Vasectomized rabbits injected with complete Freund's adjuvant or mumps virus show a higher incidence, earlier appearance, higher titer and longer persistence of antibodies to testicular antigens. Sera from all vasectomized animals tested to date have been negative for antibodies to steroid-producing cells.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract should provide important information on possible autoimmune reactions to steroid-producing and other tissues after vasectomy in animals and men.

Proposed Course: The studies of sera from vasectomized men will be extended to larger numbers, including individuals with a family history of autoimmune disease. Animal studies of humoral and cell-mediated immune responses to vasectomy and characterization of testicular antigens will be continued and these studies will be extended to investigations of BUF rats and A line beagles, strains which are highly susceptible to spontaneous autoimmune disease.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Endocrine Changes in Vasectomized Men
Contractor: University of Texas Medical School at Houston
Money Allocated: \$185,000 (FY 1974 funds for two years)

Objectives: The purpose of this program is to conduct a prospective study of endocrine function in men undergoing bilateral vasectomy. Plasma levels of testosterone, estradiol, 20 α -dihydroprogesterone, FSH, and LH are being determined in approximately 200 men prior to vasectomy and at 1 and 6 weeks and 3, 6, 12, 24, and in some cases 36 months after vasectomy.

Major Findings: One hundred and sixty patients have been enrolled and successfully followed at the intervals planned. Fifty of these patients are also being studied for immunologic changes under a separate contract with Baylor University. Preliminary endocrine data have demonstrated no adverse changes in hormone levels at intervals up to one year after vasectomy. The data do suggest that levels of testosterone are significantly higher and levels of estradiol are somewhat lower one year after vasectomy than at the time of surgery or in the early post-operative months. This effect may not persist when larger numbers are available at the longer follow-up intervals, or it may be attributable to psychological stress in connection with the surgical procedure. If it continues to be seen when more data are available, plasma samples will be analyzed for levels of adrenal cortical steroids.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Data are needed on the endocrine effects of vasectomy since such effects would not necessarily be expressed somatically or behaviorally. This study will provide longitudinal data on levels of testosterone, estradiol, FSH, LH, and 20 α -dihydroprogesterone. Levels of the latter steroid may be indicative of testicular damage if this should occur.

Proposed Course: It is expected that this study will be continued for two to three additional years in order to obtain and analyze 2-year follow-up data on 200 vasectomy patients. Approximately 100 of these patients will reach the 3-year postoperative interval and will also be studied at that time.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Ultrastructural and Immunochemical Studies of Sperm Antigens
Involved in the Autoimmune Response in Vasectomized Mammals
Contractor: University of Miami
Money Allocated: \$27,534 (FY 1974 Funds)

Objectives: The original purposes of this contract were to study the effects of antisperm autoantibodies on sperm fertility, the localization of these antibodies in sperm and testicular tissue, and the rôle of permeability of the blood-testis barrier in the development of an immune response to vasectomy. However, because of personnel changes, the studies have concentrated on investigations of changes in the seminiferous epithelium and the permeability of the blood-testis barrier in immunized and vasectomized animals.

Major Findings: Histologic and electron microscopic studies of the seminiferous epithelium have been conducted in guinea pigs sacrificed at 10, 20, 30, and 60 days and 6 months after unilateral vasectomy, immunization with testicular homogenates, injection of Freund's adjuvant, or sham operation. In the vasectomized animals varying degrees of damage were found in the testes on the vasectomized side, but lesions were rare in the contralateral testes. No testicular lesions were seen in animals sacrificed at 6 months after vasectomy. All guinea pigs immunized with testicular homogenates showed marked aspermatogenesis at 10-60 days and lesser degrees of damage at 6 months. Freund's adjuvant did not appear to induce further damage to the testes of vasectomized guinea pigs and Freund's adjuvant alone caused no histologic lesions.

Electron microscopic studies of the permeability of the blood-testis barrier to lanthanum have been conducted in control guinea pigs and guinea pigs immunized with testicular homogenate, and results suggest increased permeability of the barrier in the immunized animals.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies supported by this contract should help elucidate the mechanisms involved in an autoimmune response to vasectomy.

Proposed Course: Studies under this contract were terminated during FY 1974.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Sperm Immunology
Contractor: Netherlands Cancer Institute
Money Allocated: \$61,930 (FY 1974 Funds)

Objectives: This contract supports work on isolation and characterization of autoantigens from human spermatozoa and the development of techniques for assessing immune responses to sperm antigens. Studies are conducted using sera from naturally infertile and vasectomized men. Previously planned investigations of the immunoglobulin classes of sperm agglutinating antibodies have been deferred in order to concentrate on antigen characterization and methodological work.

Major Findings: Further investigations have been conducted on the swollen sperm head antigen identified last year on human sperm treated with trypsin and dithiothreitol. Methods of detecting and solubilizing the antigen and data on the occurrence of antibodies against it in human sera have been reported (Clin. Exp. Immunol., 16: 63-76, 1974). The antigen has been purified by a variety of chromatographic and electrophoretic procedures. It is a strongly basic protein of low molecular weight. Antibodies against this nuclear antigen have been detected in low titer in vasectomized men.

In methodological studies under this contract, a number of modifications of the macroscopic Kibrick sperm agglutination assay have been tested for use on a micro scale and comparative studies of macroscopic and microscopic assays are in progress. Preliminary data suggest that there may be some differences in the antibodies detected by the two techniques.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number supported by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Methodological work under this contract should contribute significant practical improvements in assay methods for humoral and cell-mediated immunity to sperm. The isolation and characterization of specific sperm antigens has been a critical need in order to improve the quality and precision of data from studies in reproductive immunology, and the isolation of defined antigens may also lead to the development of new contraceptive approaches.

Proposed Course: The swollen sperm head antigen will be further characterized, with studies of its amino acid composition and end groups. The occurrence and characteristics of antibodies to this antigen will be studied and possible cross-reactions with other nuclear antigens will be investigated. The micro

sperm agglutination assay will be further validated and attempts will be made to develop an assay for cellular immunity to spermatozoa.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Autoimmune Phenomena and Mechanisms in Human Vasectomy
Contractor: University of Cincinnati Medical Center
Money Allocated: \$149,500 (FY 1974 Funds)

Objectives: The purpose of this contract is to evaluate alterations in immunologic responses, blood coagulation mechanisms, and medical status in 50 vasectomized men. Tests are conducted before and at intervals after vasectomy to assess the development of circulating antibodies and delayed hypersensitivity to sperm and seminal fluid antigens and of circulating immune complexes, alterations in humoral and cellular immune responsiveness, the occurrence of antinuclear antibodies, rheumatoid factor and other indicators of autoimmune disease, and changes in factors involved in blood coagulation. Clinical examinations and medical histories are also obtained at each visit.

Major Findings: Forty-one of the planned 50 patients have been enrolled and follow-up examinations have been conducted as planned. Eight of the subjects have been seen at twelve months after vasectomy. Early data are available for most of the parameters to be measured. In general, except for the development of sperm agglutinating antibodies in about two thirds of the subjects, the results available thus far do not demonstrate striking immunologic changes in post-vasectomy specimens as compared to pre-vasectomy specimens from the same subjects. However, some changes of uncertain significance have been seen, including marked fluctuation in values for migration inhibition factor in response to genital antigens, variability in levels of the third component of complement, and occasional immunoglobulin levels outside the normal range. In the blood coagulation tests, mean fibrinogen levels were significantly elevated after vasectomy and post-vasectomy levels of circulating fibrin and fibrinogen/fibrin split products were elevated in some of the patients as compared with pre-vasectomy levels in the same patients. At this time it is not clear whether these results may be due to inherent vagaries in the assays, to individual variability over time, or to the effects of vasectomy. Data from the coagulation study also may reflect the effects of any minor surgery. Serial studies of normal controls are needed to resolve these questions.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. A number of contracts have been initiated by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. This contract is one of several projects involving intensive clinical study of vasectomized men and it should provide important information on alterations in the immunologic and hemostasis systems and in medical status after surgical sterilization.

Proposed Course: The current studies will be continued in the subjects already enrolled and in 20 new vasectomy patients and 20 matched normal controls. Some refinements in technical procedures will be introduced and levels of secretory IgA in seminal fluid will be determined as a measure of local immune response.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Immunopathologic Consequences of Vasectomy
Contractor: University of New Mexico
Money Allocated: \$54,520 (FY 1974 Funds)

Objectives: This contract provides for studies of the effects of vasectomy in about 500 human subjects and in experimental animals. Sera are monitored for antibodies to sperm. Immunoglobulin classes of the antibodies, their cross-reactivity with other tissues, and their ability to activate complement components are being determined. Assays are run for cell-mediated hypersensitivity. The patients are followed for symptoms of a possible autoimmune response to sperm. Pathological lesions are sought in the testis and other organs of vasectomized animals and attempts will be made to induce similar lesions by passive and adoptive transfer. The relationship between appearance of antisperm antibodies, degenerative changes in the testis, and sperm granuloma formation will be examined in vasectomized animals and the role of an immune component in the sperm granuloma reaction to α -chlorhydrin will be studied in rats with induced tolerance to sperm.

Major Findings: Sera of 80 patients have been tested for antisperm antibodies before vasectomy and 2 and 9 months after the operation. Indirect immunofluorescence techniques, which are more sensitive than sperm agglutination or sperm immobilization, have demonstrated two kinds of antibodies. The first type was found in low titer in 50% of the patients before vasectomy, increased in titer 2 months after surgery and showed little further change at 9 months; this type of antibody is directed to antigens of the sperm acrosome, equatorial segment and postacrosomal region. The second type of antibody was found in only 2-3% of patients before vasectomy and increased in incidence to 25% of patients 2 months after vasectomy and 50% 9 months after vasectomy; this type of antibody is directed to antigens in the sperm tail or occasionally to discrete areas of the acrosome. Both antibodies are present as IgG and IgM classes and are sperm specific. Very preliminary data also suggest the development of low titers of antinuclear antibodies of IgM class in several patients 2 months after vasectomy; these data are being checked in further studies of patients' sera and in immunized animals.

Histopathologic studies in bilaterally vasoligated and sham operated rats have documented the universal occurrence of sperm granulomas in the cauda epididymis and vas deferens within 3-4 months after vasoligation in this species. These changes were rarely accompanied by testicular pathology; aspermatogenesis was seen in only 4 of 53 animals after vasoligation and probably resulted from damage to adjacent structures or retention of the testis in the abdominal cavity. Sperm granulomas are composed of sperm surrounded by histiocytes and lymphocytes, and may well be a source of stimulation of an immune response to sperm.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world but there is a possibility that complex physiological and immunologic effects

may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number supported by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract should provide valuable new information on autoimmune reactions to vasectomy and the pathogenetic mechanisms which may be involved.

Proposed Course: It is expected that investigations under this contract will be continued for a number of years.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: USC Collaborative Study of the Effects of Vasectomy
Contractor: University of Southern California Medical Center
Money Allocated: \$337,000 (FY 1973 funds for 18 months)

Objectives: The purpose of this study is to conduct a pilot evaluation of the effects of vasectomy in 50 patients and 50 matched controls who will be studied prospectively. The evaluation will include medical history, physical examination, psychological evaluation, clinical laboratory data, humoral and cellular immune alterations, and hormonal changes. The first contract period is intended to support a pilot study during which the administrative operations and laboratory data will be evaluated and plans will be made for appropriate modifications and possible expansion of the study in subsequent years.

Major Findings: Forty-three patients and 23 control subjects have been enrolled in the study and the earliest enrollees are now approaching the 6-month evaluation. Difficulties encountered in recruiting control subjects appear to have been overcome. The study is proceeding according to plan but it is too early for significant findings.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but complex physiological and immunological effects may result from this procedure. This project is one of a number initiated by the Center to define the long term consequences of vasectomy, the mechanisms involved in these alterations, and their relation to the possible occurrence of systemic complications. This project will provide a comprehensive evaluation of medical, immunologic, endocrine, and psychological alterations in a small group of men on a pilot basis. Data from this study and from other contracts in this program will provide needed practical information for planning subsequent prospective studies.

Proposed Course: This 18-month pilot project was initiated at the end of FY 1973 and is expected to be concluded in mid-1975, although some additional time may be required for 12-month follow up on all subjects. Plans for continuation or expansion of the study will depend on data available at the conclusion of the pilot study.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Immunologic and Morphologic Effects of Vasectomy in Monkeys
Contractor: Tulane University
Money Allocated: \$278,335 (FY 1973 Funds for Two Years)

Objectives: The purposes of this contract are to study the immunologic responses and morphological changes in testis, epididymis and vas deferens in vasectomized monkeys and to determine the role of extravasation of sperm in the initiation of an immune response to vasectomy. The effects of three surgical techniques varying in degree of sperm spillage will be compared. Immunologic tests include circulating antibodies to reproductive antigens, their immunoglobulin classes and cross reactivity with other tissues, and cell-mediated immune responses. Morphologic changes in testis, epididymis and vas deferens will be sought by light and electron microscopy, and data will be correlated with results of immunologic studies in the same animals.

Major Findings: This study is proceeding according to plan but it is too early for any significant findings from the longitudinal studies of vasectomized monkeys. However, preliminary work has included an effort to develop a new assay for antisperm antibodies which will be more sensitive and less subjective than sperm agglutination assays. A passive hemagglutination assay has been developed using antigens extracted from subcellular sperm fractions adsorbed to sheep red blood cells. This assay has significant potential as a standardized method for detecting and measuring antisperm antibodies and it also can be used in an inhibition assay to test for cross reactions. Studies with this assay may lead to localization and identification of specific antigenic sites of sperm at the ultrastructural level, thus greatly increasing the specificity of studies in reproductive immunology.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there may be complex physiologic and immunologic effects as a result of this procedure. This project is one of a group initiated by the Center to define the long-term consequences of vasectomy, the mechanisms involved in the development of these alterations, and their relation to the possible occurrence of systemic complications. This project will provide a thorough evaluation of morphologic changes in the vasectomized monkey and correlation between morphologic and immune alterations, and should also provide information on the role of variations in surgical technique in the initiation of an immune reaction. An added bonus has been the development of a significant new assay for circulating antibodies to sperm.

Proposed Course: This project is expected to require at least another year to complete.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Immune Alterations Associated with Vasectomy
Contractor: Baylor College of Medicine
Money Allocated: \$80,000 (FY 1974 Funds)

Objectives: This contract supports prospective studies of the incidence and progress of immune alterations in vasectomized men. Studies are being conducted before and at intervals after vasectomy in 48 men who are also under study for endocrine alterations under a separate contract with the University of Texas. Tests are being conducted of sperm immobilizing and sperm agglutinating antibodies, HLA antigens, serum immunoglobulins, autoimmune antibodies, and cellular immunity to reproductive antigens. Data from three standardized sperm serology assays are being correlated with other data on the same subjects in an effort to determine the functional significance of positive serological reactions. Methods are being developed for measuring cellular immunity to sperm.

Major Findings: This project has been in operation for less than a year and only preliminary results are now available. Positive sperm agglutination and sperm immobilization tests after vasectomy have been recorded in substantial numbers of individuals and it has been demonstrated that the two agglutination assays employed (Franklin-Dukes and Kibrick) are more sensitive than the sperm immobilization assay in revealing positive conversions after surgery. A transient and thus far unexplained decrease in all classes of serum immunoglobulins measured (IgG, IgM, IgA and IgE) has been observed in a substantial proportion of the patients in the early post-operative period as compared with pre-operative levels for the same patients. An unexpectedly high percentage of positive results has been encountered in pre-vasectomy lymphocyte microcytotoxicity tests with pooled seminal antigens, and no consistent pattern of responsiveness has emerged in serial tests in the lymphocyte transformation assay.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but complex physiological and immunological effects may result from this procedure. This contract is one of a number initiated by the Center to define the long term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Data from this project will provide detailed information on immunological changes in vasectomized men and also a careful evaluation of the significance of three commonly used serological assays for circulating antibodies to sperm.

Proposed Course: It is expected that the patients presently enrolled will continue in the study and the population will be expanded to include an additional fifty subjects. Work in the second contract year will be focussed on antisperm antibodies, immunoglobulin profiles, the occurrence of autoantibodies, and the development of a ⁵¹Cr-release technique for measuring cellular immunity to sperm.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Peripheral Metabolic Effects of the Ethynyl Estrogens in Subhuman Primates and Canines as Compared to Man
Contractor: Southwest Foundation for Research and Education
Money Allocated: \$138,603 (FY 1974 Funds)

Objectives: Studies supported by this contract are determining the effects of two dosage levels of ethynyl estradiol and mestranol, alone and in combination with norethindrone, megestrol and norgestrel, on certain metabolic functions in baboons and beagles. The results will be compared with similar data being obtained for human subjects under support from other sources. The effects of the steroids on glucose tolerance, plasma lipoproteins, and plasma cortisol, testosterone and androstenedione are being determined in both species. Effects on serum bilirubin, fibrinogen, alkaline phosphatase, SGOT, tryptophan, ceruloplasmin, thyroxine, and steroid-binding protein are being determined in the baboons.

Major Findings: The protocol for this project requires studies of the effects of estrogen alone and then of progestin-estrogen combinations in 30 beagles and 30 baboons, followed by replication of these studies in a second group of 30 animals of each species. The first replication has been completed and the preliminary data suggest significant biological differences between baboons and beagles in the effects of estrogen treatment on lipoprotein patterns, carbohydrate metabolism, and circulating hormone levels. However, data from the second replication which is now in progress are required before valid conclusions can be drawn.

Significance to Biomedical Research and Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval of these drugs for clinical use. However, interpretation of the results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. Data from this study will be important in determining whether beagles or baboons can be used as appropriate animal models for predicting the effects of contraceptive steroids on these hormonal and metabolic parameters in women.

Proposed Course: It is expected that another year of support will be required to complete this study.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Biliary and Urinary Excretion, Enterohepatic Circulation, and Hepatic Effects of Contraceptive Steroids
Contractor: Health Research, Inc., Roswell Park Division
Money Allocated: \$80,000 (FY 1974 Funds)

Objectives: The metabolism of six contraceptive steroids, including representatives of the 17 α -acetoxyprogesterone series and of the 19-nor steroids are being studied in baboons and in human subjects by administering tracer amounts of radiolabeled steroids and determining urinary and biliary excretion of radioactivity and enterohepatic circulation and isolating and identifying urinary and biliary metabolites. In baboons, the effects of pharmacological doses of the steroids on biliary excretion, enterohepatic circulation, liver function, and damage to bile canaliculi and hepatic cells is also being determined.

Major Findings: Studies of the metabolism of progesterone, norgestrel, norethindrone and dimethisterone have been carried out in baboons with bile duct fistulas, and norethindrone has also been studied in human subjects with bile drainage. The progesterone studies used 21-¹⁴C-progesterone mixed with 7-³H-progesterone and ³H/¹⁴C ratios were determined in free, glucuronide, sulfate and diconjugated steroids from urine and feces. Ninety percent of the radioactive steroid was excreted as conjugates. Three major peaks have been separated from the glucuronide fraction, one representing hydroxylated metabolites, one androgen metabolites, and one with the same isotope ratio as the starting material. In studies of norgestrel, most of the metabolites in urine and bile were glucosiduronides; these have been further fractionated into five major components which have been partially characterized. Studies of norethindrone in human subjects indicate that this compound is heavily hydroxylated, with about 60% of the conjugates appearing as glucosiduronides and 40% as sulfates or sulfoglucosidurides; the nature of the metabolites is different in urine and bile, suggesting extensive enterohepatic circulation. Preliminary studies of norethindrone in baboons suggest a complex pattern of metabolites in urine and a relatively simple pattern in bile, comparable to patterns observed in man. Dimethisterone is apparently not heavily metabolized in the baboon and is conjugated primarily with glucosiduronic acid. Ultrastructural studies of liver biopsies from baboons chronically treated with norethindrone demonstrate definite changes which revert to normal after discontinuance of the steroid.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions may not be readily extrapolated to man. Other species may be more suitable than those presently used for testing these drugs, and the baboon, which is phylogenetically closer

to man, may prove to be an excellent choice. This contract is one of several supported by the Center to study the metabolism and effects of contraceptive steroids in the baboon. It should provide significant data on the suitability of the baboon as a test animal for these drugs.

Proposed Course: These and related studies will probably be supported for several additional years.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Contraceptive Steroids in Beagle Dogs
Contractor: Cornell University College of Agriculture and Life Sciences
Money Allocated: \$108,500 (FY 1974 Funds for 15 months)

Objectives: The purposes of this contract are 1) to develop radioimmunoassay methods for LH, FSH, prolactin, estrone and estradiol and protein binding assays for measurement of progesterone and glucocorticoids in plasma samples from beagle dogs, 2) to use these assays to determine levels of polypeptide and steroid hormones in the normal reproductive cycle of the beagle, and 3) to study the effects of contraceptive steroids on endocrine organ function in this species.

Major Findings: Satisfactory assays for plasma progesterone, estrogens, corticoids and LH have been established and validated in beagle dogs. Plasma levels of these hormones have been measured during estrous cycles and in pregnancy. The usual mammalian estrogen - LH - progesterone sequence at estrus has been confirmed in this animal. An interesting finding is that sex steroid levels do not rise during pregnancy and that parturition is immediately preceded by a rise in corticoids and a fall in progesterone.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. This contract has developed methods for use in studies of the effects of contraceptive steroids on endogenous hormones in beagle dogs. The project is expected to contribute to understanding of the biological and pathological effects of contraceptive steroids in a species which is a required test animal for these drugs.

Proposed Course: Studies under this contract are now being extended to determine the effects of medroxyprogesterone acetate on plasma hormone levels, endocrine organ function and mammary gland pathology in the beagle. Work will also be continued on the development of assays for FSH and prolactin.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Metabolism of Contraceptive Synthetic Estrogens
Contractor: Loyola University Medical Center
Money Allocated: \$36,600 (FY 1973 Funds)

Objectives: This contract has supported studies of the metabolism of ethinyl estradiol and mestranol in baboons. Tracer doses of radiolabeled steroid are administered during the proliferative phase and again during the luteal phase of the estrous cycle, and blood and urine samples are obtained for metabolic studies. After an interval without medication, the animals have been given a combination type steroid contraceptive orally for two to three consecutive cycles, after which the tracer studies are repeated. The disappearance of radioactivity and of the administered drug from blood is being measured for calculation of metabolic clearance rates and plasma half-lives. Urinary metabolites are being isolated by chromatographic methods and hydrolysis of conjugates.

Major Findings: Techniques have been established for following a sequence of menstrual cycles in the baboon, correlating sex skin changes with vaginal smears and plasma progesterone levels. Techniques have also been devised for extracting and isolating radioactive ethinyl estradiol and mestranol from baboon plasma and correcting for procedural losses. Preliminary data from kinetic studies of ethinyl estradiol suggest that uptake of the steroid by tissues may be significantly different in pre- and post-ovulatory phases of the cycle, but the differences observed may be due to lack of precision of the methods or study of insufficiently frequent samples. Crude fractionations of urinary metabolites have been accomplished and 2-hydroxy and 2-keto derivatives of ethinyl estradiol and 2-hydroxy mestranol have been tentatively identified. Preliminary extractions of pooled plasma have demonstrated that the majority of the conjugates of ethinyl estradiol can be extracted with ethyl acetone and released by solvolysis.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to their approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot be readily extrapolated to man. Specifically, significant differences in absorption, metabolism, excretion and biologic potency may account for effects which may be unique to the species under evaluation and thus not relevant to man. Other species may be more suitable than those presently used for testing these drugs, and the baboon, which is phylogenetically close to man, may prove to be an excellent choice. Studies of the metabolism and effects of contraceptive steroids in the baboon may aid in determining its suitability as a test animal for these drugs.

Proposed Course: Studies under this contract will be terminated at the end of the current fiscal year.

NICHHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Central Repository for Tissue Evaluation of Contraceptive Steroids

Contractor: Universities Associated for Research and Education in Pathology, Inc. and Armed Forces Institute of Pathology

Money Allocated: \$137,500 (FY 1974 Funds)

Objectives: The purpose of this contract is to establish a central repository for tissue specimens from animals receiving hormonal contraceptive agents and conduct pathologic evaluation of the tissues. Contributions of tissue specimens have been solicited from pharmaceutical companies and other sources. Standardized procedures are used in processing specimens, and interpretation of pathology will involve consultative circulation through appropriate divisions of the Armed Forces Institute of Pathology.

Major Findings: Major emphasis in this project to date has been on the acquisition of tissues from long-term toxicity tests of contraceptive steroids. Most of these tests are conducted by pharmaceutical companies or by contractors for the Food and Drug Administration. Specimens and related data have now been obtained on 218 dogs, 111 monkeys and 951 rats treated with many of the more important contraceptive steroids, and specimens of normal mammary tissues and spontaneous mammary lesions in beagle dogs have been obtained from several beagle colonies. Histological processing has been completed on most of the dog and monkey specimens received thus far. Primary effort during the next year will be devoted to pathologic evaluation, particularly of mammary tissues from beagle dogs.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use. A major aspect of this testing is long term study in beagle dogs, a species which is unusually prone to development of breast nodules. Several promising contraceptive steroids have been removed from the market when breast nodules developed in beagles treated with these compounds, but there is little information on the pathology of the beagle lesions or their possible relationship to human cancer. This contract was awarded to meet the need for central, expert and unbiased review of tissues obtained from various laboratories and processed with standardized methods; it is expected that the nature of these lesions may thus be determined.

Proposed Course: This contract is expected to be renewed annually for several years.

NICHD ANNUAL REPORT

July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Comparative Pharmacology of Steroid Contraceptive Drugs
Contractor: Mario Negri Institute of Pharmacological Research
Money Allocated: \$153,300 (FY 1974 Funds)

Objectives: This contract supports studies in five major areas: 1) The effects of steroid contraceptive drugs on neurochemical mediators in the brain and target organs; 2) effects on immunological responsiveness and 3) the invasiveness of cancer; 4) effects on lipid metabolism and lipolysis in adipose tissue; and 5) the interaction of steroid contraceptive agents with other drugs. In all of these investigations, data are correlated with antifertility effects in the test species. Studies are conducted in mice, rats, guinea pigs and rabbits. The animals receive one of three drug combinations administered orally (norethynodrel plus mestranol, norethindrone plus mestranol, and lynestrenol plus mestranol), and in some experiments additional animals receive the individual steroids alone. A range of dosages is used in both acute and chronic studies and dose-response effects are being determined.

Major Findings: The effects of acute and chronic treatment with steroid contraceptives on levels of neurochemical transmitters and their precursors and activities of enzymes involved in synthesis of these compounds have been measured in several areas of rat and mouse brain and in uterus and ovaries. Significant changes are reported in levels and turnover of some brain monoamines. The effects seen are not marked and are apparently not related to antifertility action since reductions in serotonin by lesions of the mid-brain raphe and reductions in noradrenalin by intraventricular injection of 6-hydroxydopamine did not affect antifertility action of contraceptive steroids.

Studies of the effects of steroid contraceptives on immune responsiveness have been conducted in several test systems. When a combination of lynestrenol plus mestranol was given to rats and mice at various dosages and schedules, no effects were seen on the number of antibody-producing spleen cells in response to administration of sheep red blood cells. Similar tests with norethindrone plus mestranol demonstrate some suppression of antibody-forming cells, particularly in mice, and these effects are seen only with the combination drug, not with either steroid alone. Studies of the course of experimental allergic encephalomyelitis in rats have demonstrated almost complete inhibition of the disease by treatment with either steroid combination, indicating suppression of predominantly cell-mediated immune responsiveness. Two other experimental models of autoimmune disease (thyroiditis and adrenalitis) are being established for testing effects of contraceptive steroids. Effects of the steroids on in vitro transformation of lymphocytes by phytohemagglutinin are being tested as another correlate of cell-mediated immune responsiveness, and preliminary data suggest decreased

responses in rats treated with lynestrenol and mestranol.

The effects of contraceptive steroids on cancer growth, dissemination and metastasis have been studied in three test systems. Lynestrenol plus mestranol did not significantly affect survival time, tumor growth or dissemination or the growth of metastases in mice bearing either of three transplanted tumors. Norethindrone plus mestranol caused a slight inhibitory effect in one system and a significant increase in metastasis in another.

Studies have been conducted on lipid fractions in plasma, liver, heart, ovary, uterus and adrenals during the estrous cycle and after acute and chronic treatment with various combinations and dosages of contraceptive steroids. Consistent increases in plasma triglycerides and decreases in plasma corticosterone and plasma and adrenal cholesterol were demonstrated in rats even at dosages much below the antifertility level. The effects are mainly due to the estrogen component and are not clearly related to dosage. They do not occur in mice but mice do show reduction in liver triglycerides. Basal lipolysis of adipose tissue is reduced by chronic steroid treatment in the rat but not in the mouse, and noradrenalin-stimulated lipolysis was unaffected in both species.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use. However, interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. This contract supports a wide range of dose-response studies of the effects of contraceptive steroids in laboratory animals; much of the work is imaginative and is likely to yield valuable new information on the effects of these widely used drugs.

Proposed Course: It is expected that work under this contract will be continued for several years.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: The Baboon: An Animal Model for the Study of Contraceptive Steroids
Contractor: New York Hospital-Cornell Medical Center
Money Allocated: \$8,653 (FY 1974 Funds for Two Months)

Objectives: The original purpose of this contract was to develop a modification of the Thomas duodenal cannula for permanent implantation in the baboon and to use this animal model in studies of the effects of contraceptive steroids on biliary function and in collaborative studies of the metabolism of contraceptive steroids. In view of difficulties encountered with the duodenal cannula, this has been replaced with a procedure involving permanent attachment of the gallbladder to the abdominal wall.

Major Findings: Surgical complications and a high post-operative mortality rate led to discontinuance of attempts to develop a modified duodenal cannula for use in the baboon. An alternative gallbladder pexy procedure has been developed, involving suturing the fundus of the gallbladder to the anterior abdominal wall. Cholecystograms after recovery from surgery demonstrate that the gallbladder fills and empties normally with this preparation and bile samples can be obtained at any time through a small hypodermic needle inserted through the skin into the gallbladder. Several baboons prepared in this way have now survived for more than a year. Studies of bile acid pool size and turnover and the effects thereon of contraceptive progestins and estrogens are now in progress.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. Other species may be more suitable than those presently used for testing these drugs, and the baboon, which is phylogenetically close to man, may prove to be an excellent choice. This contract is one of several supported by the Center to study the metabolism and effects of the contraceptive steroids in the baboon and determine its suitability as a test animal for these drugs. Studies supported by this contract should provide significant data on biliary effects of the steroids and also facilitate studies of their metabolism by other investigators. The studies of effects on biliary function may also contribute valuable insights into mechanisms responsible for the reported increased incidence of gallstones in women taking steroid contraceptives.

Proposed Course: Now that a successful gallbladder preparation has been developed for studies in the baboon, it is expected that this contract will be renewed for one or more subsequent years to support further studies of the effects of contraceptive steroids on biliary function and collaboration with others in investigations of the metabolism of these agents.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Pharmacopathologic Effects of a 17α -Hydroxyprogesterone Derivative in Beagle Bitches
Contractor: University of Rochester School of Medicine and Dentistry
Money Allocated: \$166,000 (FY 1974 Funds)

Objectives: This contract supports studies of the effects of medroxyprogesterone acetate (MPA) on uterine and mammary gland structure and function in beagles in an attempt to explain the unusual sensitivity of this species to derivatives of 17α -hydroxyprogesterone. The effects of three dosages of MPA on morphological, histochemical and biochemical characteristics of mammary glands and uteri are being investigated. Glands and uteri are removed for study at intervals of 3-4 months for a period of three years, and complete necropsy examinations will be conducted after 12, 24 and 36 months of treatment.

Major Findings: Studies under this contract are proceeding essentially as planned but it is too early for any significant findings on the effects of medroxyprogesterone acetate on mammary and uterine tissues of the beagle dog. The 104 dogs under study are divided into four groups receiving 3.0, 1.5, and 0.3 mg MPA per kilogram every three months and a control group receiving no steroid. Ovulation has been completely suppressed by the highest dosage, markedly delayed in the intermediate dosage group, and unaffected by the lowest dosage. Histopathologic and biochemical evaluation of mammary and uterine tissues from early treatment periods and from control animals in various stages of the reproductive cycle are in progress and plans for computer analysis of the data are being developed. In view of data on the time required for development of beagle mammary nodules in other toxicity studies, the surgery schedule is being revised to provide for a maximum exposure period of three years.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. Long-term studies in beagle dogs are included in the required testing, and several promising contraceptive agents have been removed from the market when mammary nodules occurred in the beagles, a species which is unusually prone to development of breast lesions. Work under this contract should help clarify the nature of the beagle nodules and their possible relationship to breast cancer in human subjects.

Proposed Course: Two additional years will probably be required to complete this study.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Commonly Used Drugs on the Metabolism of Ethynyl Estradiol and Mestranol
Contractor: Worcester Foundation for Experimental Biology
Money Allocated: \$24,025 (FY 1974 Funds)

Objectives: Studies are being conducted to determine the effects of acute and chronic treatment with commonly used drugs on the metabolism of contraceptive estrogens in laboratory animals. Drugs to be studied are selected from among such widely used agents as phenobarbital, aspirin, secobarbital, disulfiram, and chlorpromazine. In vitro studies of steroid demethylation and 2-hydroxylation are conducted in rat liver microsomal preparations, possible drug-induced alterations in other metabolic pathways are being sought, and correlations between data obtained in microsomal preparations and in vivo experiments are being determined.

Major Findings: Incubation conditions and methods for subsequent work-up have been perfected for studies of the metabolism of mestranol and ethynyl estradiol in microsomal preparations. 2-³H-mestranol and 2-³H-ethynyl estradiol have been synthesized and the location of the label at the 2 position confirmed. The demethylation and subsequent 2-hydroxylation of mestranol have been studied in liver microsomal preparations from untreated rats and from rats pretreated with phenobarbital. Incubations were performed with 6,7-³H-mestranol and ¹⁴C-(methoxyl)-mestranol and analyzed for amounts of ethynyl estradiol and formaldehyde produced; in similar incubations with 2-³H- and 4-¹⁴C-mestranol, yields of ethynyl estradiol and tritiated water were determined. In both series of experiments recovery of unchanged mestranol was also quantitated and material balances demonstrate that the methodology is excellent. The data indicate that phenobarbital treatment enhances both the demethylation and the 2-hydroxylation reactions. The administered radioactivity is essentially completely accounted for in the control studies but about 10% is unaccounted for after phenobarbital treatment, suggesting the possibility of enhancement of other metabolic pathway(s), perhaps metabolism prior to demethylation.

Significance to Biomedical Research and the Program of the Institute: The problem of possible effects of commonly used drugs on the metabolism of steroidal contraceptives in humans is an important one, and these studies in drug-treated animals should contribute significant information on effects which may be expected in man.

Proposed Course: These and related studies are expected to be continued for several years.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: A Study of the Metabolism of Steroidal Oral Contraceptives
Contractor: Medical College of Georgia
Money Allocated: \$80,455 (FY 74 Funds for 18 Months)

Objectives: The purpose of this contract is to obtain information on the plasma half-life, metabolic clearance rate (MCR), and urinary and circulating metabolites of norethindrone and mestranol in normal women of reproductive age and on the effects of chronic use of steroid contraceptives on these parameters.

Major Findings: Plasma half-lives and metabolic clearance rates for norethindrone have been determined in 8 patients prior to contraceptive use and these studies have been repeated in 6 of the subjects after chronic administration of Norlutin (norethindrone 2.5 mg). Pretreatment studies for mestranol have been conducted in 7 patients and these have been repeated in 6 of the subjects after chronic administration of Ortho-Novum SQ (80 µg mestranol plus 2 mg norethindrone). The data thus far indicate no statistically significant increase in MCR after contraceptive usage, and thus no induction of steroid-metabolizing enzymes, although a slight increase would be indicated for norethindrone if one of the values were not considered. Although the administered steroid disappears rapidly from blood, large amounts of radioactivity remain in plasma, presumably as conjugates with much longer half-lives. The half-lives of these metabolites are under study and their possible accumulation in chronic users of oral contraceptives is being investigated.

Urines from these patients have been collected and radioactivity has been determined in urine and in free steroid, sulfate, and glucuronide fractions. Preliminary work has been done on separating and characterizing urinary metabolites using paper and thin layer chromatography and gas-liquid chromatography with mass spectrometry. Further progress in characterizing blood and urinary metabolites is expected during the current contract period.

Significance to Biomedical Research and the Program of the Institute:

Detailed knowledge of the metabolism of contraceptive drugs is necessary to understand the basic pharmacology of these agents and their possible long-term effects. Studies conducted under this contract will provide much needed metabolic data on two of the most commonly used contraceptive steroids in normal women of reproductive age.

Proposed Course: This project is expected to continue for several additional years in order to provide complete metabolic data on mestranol and norethindrone.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Metabolism and Metabolic Effects of Steroidal Oral Contraceptives
Contractor: Worcester Foundation for Experimental Biology
Money Allocated: \$87,200 (FY 1974 Funds for 18 Months)

Objectives: The current goals of this contract are to characterize the metabolism of mestranol in women who are long-term users of mestranol-containing oral contraceptives. Urinary metabolites are being isolated and identified to assess the extent of hydroxylation at C2 and C4 and of demethylation of the 3-methoxyl group and subsequent hydroxylation at C6 or C7. A radioimmunoassay is also being developed to determine plasma concentrations of mestranol and ethinyl estradiol; when this method is adequately established, it will be used to determine levels of mestranol and ethinyl estradiol in women using oral contraceptives containing these estrogens.

Major Findings: Two kinds of studies have been conducted to evaluate the possible effects of use of estrogen-containing oral contraceptives on the metabolism of the natural estrogens, estradiol and estrone. Metabolic clearance rates and initial volumes of distribution of estradiol and estrone have been determined in control women and women taking oral contraceptives containing mestranol or ethinyl estradiol. In four women taking mestranol, the dynamic parameters for estradiol were not significantly different from values for control subjects, but in seven women taking ethinyl estradiol there was a 1.5-fold increase in MCR and a 1.8-fold increase in initial volume of distribution of estradiol. There were no significant differences between metabolic clearance rates for estrone between control subjects and women taking either of the two synthetic estrogens. Studies of urinary metabolites of radiolabelled estradiol indicate that chronic use of mestranol-containing contraceptives has no significant effect on the pattern of metabolism of estradiol.

The metabolic clearance rates of the contraceptive estrogens are not significantly altered by chronic usage. Preliminary data have been obtained on the occurrence of a number of urinary metabolites of mestranol; ethinyl estradiol is the major metabolite in the glucosiduronate fraction. An antiserum with high specific activity for ethinyl estradiol and mestranol has been obtained and work toward developing a radioimmunoassay is in progress.

Significance to Biomedical Research and the Program of the Institute: Information on the metabolism and metabolic effects of the steroids used in oral contraceptives may provide significant leads as to the safety of these agents and the mechanisms for observed adverse effects.

Proposed Course: It is expected that studies under this contract will be continued for several years.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Postcontraceptive Reproduction in Man
Contractor: Albert Einstein College of Medicine
Money Allocated: \$62,375 (FY 1974 Funds)

Objectives: The current objective of this project is to complete an epidemiologic and cytogenetic study of the effects of prior contraceptive usage on spontaneous and induced abortions and newborn infants. Data on chromosome constitution and physical and developmental characteristics are being correlated with maternal contraceptive history and a number of medical and socioeconomic parameters.

Major Findings: Intake of cases and cytogenetic analyses have been completed but no findings can be expected until computer analyses of the data are completed later this year.

Significance to Biomedical Research and the Program of the Institute: Oral contraceptives are the most widely used method of contraception but there is little information on genetic effects on children conceived after use of these agents. This study should contribute significant data on the incidence of congenital abnormalities and cytogenetic aberrations in fetuses and newborns after maternal use of steroid contraceptives.

Proposed Course: Studies under this contract are expected to be completed in January, 1975.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: A comparative Study of Progestin Metabolism in Animals
Contractor: Pennsylvania State University
Money Allocated: \$395,100 (FY 1973 Funds for 15 Months)

Objectives: This contract supports comparative studies of the metabolism and biological effects of a series of progestational agents in monkeys, dogs, sheep, rats and mice. Investigations are being conducted on the effect of progestins on: 1) metabolic clearance rates of the progestins and of sex steroid hormones, 2) hepatic steroid metabolism, 3) extra-hepatic steroid metabolism in reproductive and non-reproductive tissues, and 4) various target tissues as measured by gonadotrophin secretion, activities of hepatic drug metabolizing enzymes, synandrogenic actions in liver, kidney and preputial gland, synthesis of cholesterol and bile acids, plasma and liver non-specific esterase activities, and levels of epidermal growth factor in the submaxillary gland. Studies will subsequently be extended to man. The purpose of these studies is to develop methods for predicting the effects of chronic progestin administration in man by relatively short term studies in animals.

Major Findings: Studies of metabolic clearance rates for testosterone and progesterone have been conducted in rats, dogs, sheep and monkeys. Studies of testosterone-estradiol binding globulin revealed that the monkey resembles man in response to sex hormones but that the sheep had no response during pregnancy; this latter species was dropped from the project because of this different biological response. Studies of progestin metabolism as related to bile flow have proceeded and detailed studies of the extra-hepatic clearance and metabolism of various progestins have been conducted. Studies of the binding and metabolism of progestins using the preputial gland of the rat have revealed differences in effects between the uterus and the preputial gland. A stimulating effect of progestins on kidney enzymes has been carefully characterized. It appears that the effect of progestins on liver enzymes does not correlate well with progestational activity on the uterus. The action of a variety of progestins alone and in combination with testosterone was examined using three end organs. The progestins were found to exert independent, synandrogenic, or antiandrogenic actions depending on the chemical tested and the nature of the end organ. Synthesis of radioactive medroxyprogesterone acetate and selected derivatives has proceeded.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. Studies supported by this contract will contribute much needed comparative information on the metabolism and

biological effects of progestational agents in five animal species and could well lead to the development of new and more appropriate test systems.

Proposed Course: It is expected that this contract will be renewed for several additional years in order to conduct similar studies of a series of progestational agents.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Development of Radioimmunoassays for Norethindrone, Norgestrel, Ethinyl Estradiol and Mestranol
Contractor: Roswell Park Memorial Institute
Money Allocated: \$60,000 (FY 1974 Funds)

Objectives: The purpose of this contract is to develop radioimmunoassay methods for determining the levels of two progestins (norethindrone and norgestrel) and two estrogens (ethinyl estradiol and mestranol) in blood and urine from humans, monkeys and beagle dogs. The assays will subsequently be used in studies of the absorption, metabolism, excretion, and biological effects of these contraceptive steroids in these three species.

Major Findings: The following steroid derivatives have been synthesized and conjugated to bovine serum albumin: the 3-carbomethoxyoximes of norethindrone, norgestrel, 6-oxomestranol and 6-oxoestradiol, and the hemisuccinates of 17 α -ethinyl estradiol and 11 α -hydroxyprogesterone. These steroid-protein conjugates have been injected into sheep and goats and antisera are being raised; high titers have been achieved in the antisera against ethinyl estradiol-3-BSA and estradiol-6-BSA. Methods have been developed for synthesizing large quantities of ³H-ethinyl estradiol and ³H-mestranol of high specific activity and small amounts of ³H-norethindrone of high activity have also been prepared. A radioimmunoassay for ethinyl estradiol in plasma of beagles and human has been set up using these reagents. The specificity of the antiserum is good but further work is required, particularly to reduce the blank, before this assay can be applied in metabolic studies.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use, but interpretation of the results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot be readily extrapolated to man. This project will provide assay methods for the most commonly used contraceptive steroids and thus make possible comparative studies of the absorption and distribution of these steroids in beagles, monkeys and human subjects.

Proposed Course: Development and validation of these radioimmunoassays will require at least an additional year of support.

NICHD ANNUAL REPORT

July 1, 1973 through June 30, 1974

Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: The Metabolism of Derivatives of 17 α -Acetoxyprogesterone in Beagles and Rhesus Monkeys

Contractor: University of Colorado Medical Center

Money Allocated: \$62,660 (FY 1972 Funds) plus \$3,000 (FY 1974 Funds)

Objectives: This contract supports pilot studies of the metabolism of a derivative of 17 α -acetoxyprogesterone in dogs and monkeys. The number and relative quantities of urinary and biliary or fecal metabolites of orally administered radioactive progestin are to be determined by classical chromatographic methods, with qualitative identification by gas chromatography-mass spectrometry techniques. Metabolic profiles in the two species will be compared and species differences in metabolism of the administered progestin will be identified.

Major Findings: Biliary fistules have been prepared in dogs and monkeys and labelled and unlabelled chlormadinone acetate has been administered with collection of bile, urine, and in some cases fecal samples. Most of the administered radioactivity appears in the bile of the dog and in the urine of monkeys. Bile and urine samples have been separated into free, glucuronide, and sulfate fractions. Fourteen products of chlormadinone acetate have been recognized in free and glucuronide fractions of monkey bile and urine. Higher proportions of glucuronides appear in dog bile than in monkey bile and more sulfates are present in monkey bile than in bile from the dogs. Further work toward separation and identification of individual metabolites is in progress.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use, but interpretation of the results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot be readily extrapolated to man. Metabolic studies of the 17 α acetoxyprogestins in two widely used test species should help clarify relationships between effects observed in these species and those which might occur in human subjects.

Proposed Course: The future course of this study will depend on results achieved in the next contract year.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Office of the Associate Director for Extramural Programs

Fiscal year 1974 has been unprecedented in the variety and magnitude of fluctuations in administrative guidelines received and programmatic decisions required of Institute staff. These changes have been felt in all areas - research grants, manpower training, and contracts. Efficiency of operation has been a critical factor in identifying problem areas and making recommendations in the best interests of Institute goals, maintenance of high quality scientific research teams, and meeting the requirements of a changing array of court actions. Once again the success achieved under heavy workloads has reaffirmed the strength of the NICHD system of parallel scientist administrator and grant/contract managerial staffs. Each has made vitally important contributions to decision making with efficiency, thoughtfulness, and consideration. It is doubtful that single individuals shouldering both responsibilities could have made as fully considered recommendations as these two groups working in close harmony. Once again we in the Extramural Programs wish to express our appreciation to our co-workers in Program Services.

NACHHD Council has reviewed in depth all of the Extramural Programs during this fiscal year. They have recognized and encouraged expansion of research support bearing on the Sudden Infant Death Syndrome and on aging processes. Expansion of these areas has involved more funds for research grants and for contracts. The SIDS emphasis has also resulted in a series of publications for researchers, health workers, and the public. The Council has maintained an active and interested Subcommittee on Adolescence which has worked closely with staff in identifying research needs and in encouraging NICHD to recognize adolescence as a research question touching upon interests of all program areas.

NACHHD Council has paid particular attention to program projects as components of a balanced research effort and to their scientific review within the NICHD. The Council Subcommittee on Program Projects and Centers studied the impact of the policy established by Council in June 1970 requiring full scientific evaluation of program projects and centers every three years. It was decided that this time period was too short for adequate program development between reviews. In addition, this timetable imposed a number of administrative difficulties both on the investigators and on NIH staff. Based on recommendations of this Subcommittee, the Council approved a policy change so as to require review during the fourth year of a five-year award. The Subcommittee also reviewed the impact of program projects and centers on overall funding of research. Primary emphasis in this review was given to the differing programmatic requirements of each Branch. The Subcommittee report will be reviewed by the Council in late June. Once again the inter-relatedness and close collaboration between the staffs and functions of the Extramural Programs and of Program Services were dramatically demonstrated as they provided needed information for Subcommittee consideration.

During FY '74, the Institute research review committees concentrated on program projects and center grants in the wake of the Administration's decision to phase out "old style" training grants. The PBIM and GD Review Committees have been merged into one, and renamed the Maternal and Child Health Research Committee. The four Committees met a total of ten times during the year.

This fiscal year has represented the first full 12-month period when each Extramural Program has had its own budget for direct operations including travel, publications, special consultants, purchases, and related expenses. This change has resulted in greater efficiency in general operation, more considered and focused decisions concerning priority activities, and more coherent long-term commitment of staff and resources to projects of high relevance. All of the Program Directors encourage NICHD to extend the Program budget concept to grants and contracts to further encourage thoughtful application of all mechanisms to achieving selected, previously agreed-upon goals.

During this year the Extramural Programs have welcomed two staff additions. Dr. Patsy Sampson, a psychologist, has joined the Growth and Development Branch staff to serve as the Executive Secretary of the Maternal and Child Health Research Committee; Dr. Sampson also is providing leadership for the expanding adolescent program in the GD Branch. Dr. T. Allen Merritt, a pediatrician, has joined the PBIM Branch staff where he has tackled the problems of human investigation in pregnancy and the neonatal period. It was with regret that we accepted the transfer of Dr. David L. Jofte from the Mental Retardation Branch to the National Cancer Institute.

A major staff problem increasingly confronting the Extramural Programs is the need for additional well trained full-time secretarial support. It simply is not possible to expand programming activities and to provide additional monitoring of grants and contracts without adequate secretarial backup for our scientist administrators. Furthermore, all Branches have been given full responsibility for organizing and conducting their own research conferences, a time consuming responsibility previously assumed by another office in NICHD. This has added greatly to secretarial workload. It is imperative for all Branches to be able to convert their present part-time and temporary secretarial positions to full-time permanent ones at the earliest practicable time.

Additional details of the many activities of the individual Programs are in the reports which follow.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Growth and Development Branch

The Growth and Development Branch supported approximately one-fourth of the research grants in the NICHD during FY 1974. This proportion has remained fairly constant for the past several years.

During this fiscal year the Branch sponsored four scientific conferences. The first, entitled "Adolescence in the Life Cycle" was a part of our expanded effort in the area of adolescence. Held in October, 1973, the purpose of the conference was to take a broad look at various areas of social and personality development in adolescence and at those elements of social structure which facilitate or impede transitions of adolescents into adult roles. This conference, which will be published as a book, delineated areas of program interest in adolescent socialization. The emphasis of the meeting was on the interaction between developmental processes--physical, cognitive, and emotional--and events in the life course that influence and are influenced by these processes.

Also in October of 1973, the Branch sponsored a conference on the "Role of Speech in Language." The purpose of this conference, the fifth in a series of meetings on Communication by Language, was to compare the development of language in animals and in individuals and to discuss the function of speech in language. An interdisciplinary group of scientists attempted to determine how and within what limits the acoustic signal, the articulation that produces it, and the phonetic message it conveys are related to the rest of the language system. By bringing together this group of investigators who represent a variety of biological and behavioral sciences it was felt that the meeting and the resultant publication could make a significant contribution to the scientific community as well as stimulate further research in this important area of child development.

In March, 1974, a conference entitled "The Phagocytic Cell in Host Resistance" was held by the Branch to provide a developmental focus on this important area of immunology. Discussions centered on mechanisms by which cells travel to sites where bacterial invaders or other foreign substances have been recognized by the human body and how these phagocytic cells ingest and kill the foreign invaders. A publication, the third in this series, is planned.

In May of 1974, the Growth and Development Branch co-sponsored a conference with the Fogarty International Center on "The Impact of Chronic Childhood Illness on Behavior" to assess the effect of chronic illness on the behavior of the young patient, his siblings and other members of his family. This conference was devoted to ascertaining the best methods for evaluating the impact of chronic illness. Experts in measurement methodology delivered position papers in their areas of competence and considered ways of measuring the effects of chronic illness on the personal psychological functioning and educational achievement of the young patient as well as the impact of his illness on family functioning.

A major publication entitled The Control of the Onset of Puberty was published in the Spring of 1974. This volume, which was edited by staff members, working with other non-Federal scientists, is the outcome of work by 17 interdisciplinary scientists and clinicians at a Growth and Development Branch sponsored conference. The book includes data on the central nervous system and the hypothalamic-pituitary regulation of puberty, steroid receptors and target organs during puberty, gonadal maturation, and somatic growth patterns in the human being and a variety of experimental animals. Emphasis in this text is on hypothalamus hormonal changes associated with sexual maturation and body composition at puberty, and/or disorders of puberty causing abnormal growth, and delayed or precocious puberty.

Mechanisms of Biological Growth

This program promotes research aimed at understanding the fundamental factors which initiate and control the process of orderly growth and development at the molecular and cellular levels. Concern extends in scope from studies on the molecular structure of the gene to studies on cellular metabolism, growth and differentiation. The chromosomes of each individual are encoded with all the information necessary to control his biological destiny. The realization of that destiny is determined by the transmission of this genetic information and the execution of the manifold encoded messages. How and when that information is released and transmitted determines the pattern of subsequent growth and development. The research program of this section is directed to the unraveling of this central molecular mystery.

The overall approach which unites these areas of interest is a mechanistic one, and the watchword is control. Research funded by this section is devoted to furthering our understanding of mechanisms that control the flow of biologic information at the molecular and cellular levels. Understanding the molecular controls that initiate, regulate and halt certain cellular activities at specific times during the process of development is a primary goal of this section. The number of ways growth and development might err is infinite; there is only one right way, yet this orderly way nearly always takes place. This system of molecular control continues to be of central interest.

At the complex level of information transfer within an integrated organism, the Branch supports research on the generation of pattern and form in biological systems. Studies on hormonal and neural mechanisms that control the onset of puberty and studies on the mechanisms of hormonal regulation of growth and development are examples of such work at this level of complexity. A recent publication of the Branch entitled the Control of the Onset of Puberty (M. Grumbach, G. Grave and F. Mayer, eds., Wiley Interscience, New York, 1974) considers this problem in detail.

Thyroxine and its control of differentiation and metamorphosis has intrigued generations of investigators, yet the mechanism by which thyroxine produces striking transformations remains elusive. However, one of our grantees has recently discovered the most primitive system known to respond to thyroxine, a medusa which contains only five cell types. Another grantee has just found the critical stage at which thyroxine acts to control the development of

cerebellar neurons.

More than 1800 hereditary diseases have been described in man, but the identity of the chromosomes which carry these mutated genes remains unknown in nearly all cases. Because of the long generation time in humans, much more is known of the genetic map of the mouse than of man. However, the advent of the technique of somatic cell hybridization has enabled geneticists to short-circuit man's generation time. Recently, a grantee has made use of this technique and has produced chromosomal maps for many groups of human genes. It has suddenly become possible to accomplish what seemed a hopeless task, the construction of a genetic map for man and the identification of the abnormal genes that determine human hereditary illness.

Immunologic Mechanisms and Pharmacological Studies in Development

The field of immunology has grown rapidly since the initial observation that an individual surviving an infectious disease seldom contracts that disease again during his lifetime. Work has continued rapidly and research in this area has expanded and has produced knowledge of considerable sophistication and applicability to the health needs of today. The emphasis now is on the differentiation of the cells of the immune system, the characteristics of cell membranes, activity associated with antibody formation, effector proteins and many similar problems.

For this reason the NICHD sees a continuing need to support and promote studies of infants and children elucidating the development of body defenses against infections, foreign bodies, therapeutic agents, environmental pollutants and disease. Our program focuses on the child as a developing organism, concentrating on those age dependent changes that occur as the process of maturation unfolds. Only by following this timely sequence of events can we fully appreciate a truly holistic concept of the immunologic system over the human life span.

The major emphasis of this program is on developmental studies relating maturation of the immunologic system to time related events. This is our unique approach. Attention is given to the control mechanisms and differentiative stimuli that occur and change throughout a lifetime in the developing fetus, the newborn, the infant and the older child as he progresses toward appreciation of his full immunologic potential.

This approach to human studies must be supplemented with phylogenetic studies in more primitive life forms to provide understanding of the developmental process. In addition, even though our mission is to increase knowledge of normal mechanisms, a program such as ours must support studies of clinical disease entities to provide insights into our understanding of the controls producing normal development. This is important since many immunologic diseases are confined to the childhood years and are of special importance to researchers and clinicians.

Emphasis also has been given to studies of the secretory immunologic system.

Its cells are found in the body tracts exposed to the external environment and includes the respiratory, gastrointestinal and genitourinary tracts. This system has gained notice since important antibodies and local immune defenses are now known to be present in the colostrum of human milk, secretions of the lungs and intestines and other body secretions. Previously immune competence was assessed only by determination of blood serum antibody titers against specific agents. Publication of the proceedings of an NICHD sponsored conference on this subject has already appeared in print under the title "The Secretary Immunologic System."

Since many important allergic conditions begin in childhood, studies of mechanisms involved in atopic disease have been given attention. A new class of antibody, immunoglobulin E., has been discovered and is involved in allergic reactions such as hay fever and asthma. To help focus on this area, a new publication resulting from another NICHD conference will be published early next year entitled, "The Biological Role of the Immunoglobulin E System."

An additional area of importance is the relationship between nutritive state and the immune response in man. A contract is currently being funded in this research area to spearhead effort focused on in depth immunologic testing of malnourished populations.

In January 1974 a conference entitled, "The Effects of Malnutrition and Infection During Pregnancy as Determinants of Growth and Development of the Child" was sponsored under contract by the Malnutrition Panel of the U.S.-Japan Cooperative Medical Sciences program. This conference focused on three important research areas:

1. Malnutrition during pregnancy
2. Infections during pregnancy
3. The problem of low birth weight

Specific recommendations were made by the participants regarding the need for further research. The proceedings from this meeting are being prepared for publication.

As an example of the type of research currently being funded, a group of NICHD supported scientists in San Francisco are currently preparing and supplying transfer factor for therapeutic use to induce cellular immunity in 78 patients around the world with immune deficiency diseases. Transfer factor is an extract derived from the white cells of peripheral blood. Diseases in which transfer factor has been effective in prophylaxis against infections or in therapy include the Wiskott-Aldrich Syndrome, severe combined immunodeficiency disease, mucocutaneous candidiasis, chronic active hepatitis, coccidiomycosis, dysgammaglobulinemia, Bechet's Disease, aphthous stomatitis, linear morphea, familial keratocanthoma and malignancy. Many of these diseases are almost certainly fatal without therapy. Not all patients in each disease category respond to the administration of transfer factor, and this has led to the recognition of different forms of the various diseases. It also indicates that these diseases may have different pathogenetic mechanisms although they may clinically appear similar. No adverse effect of

transfer factor has been observed and it is believed that the use of transfer factor should be further explored in treatment of diseases associated with depression of cellular immunity.

This approach to immunology should provide knowledge useful in such areas as improved medical treatment programs for infectious diseases (gram negative infections in newborns), better understanding and therapy for autoimmune disease (rheumatoid arthritis), and immune complex disease (glomerulonephritis), improved organ transplant procedures (skin grafts in burns), increased knowledge and prevention of allergic disorders (asthma, eczema), better understanding of oncogenesis and its control (neuroblastoma), knowledge useful for therapy of immune deficiency disease (agammaglobulinemias), improved immunoprophylaxis (development of new and improved vaccines), and improved pharmaceuticals made possible by knowledge of how they interact with invading organisms and the immune system to bring about the desired results.

Since research in immunology is so closely combined with clinical study and treatment of patients, emphasis will continue to be placed on problems unique to the childhood years.

Nutrition

Nutrition research supported by the Growth and Development Branch reflects the multidisciplinary implications of adequate or improper nutrient intake and utilization. Emphasis continues to be placed on the role of nutrition in physiological and intellectual development, and the important interactions among nutrition, behavior and environment. Studies of the metabolic, cellular and behavioral correlates of the initiation of obesity in infancy, childhood or adolescence are another area of program interest. Several investigators supported by the Branch are conducting diverse studies with the aim of more clearly defining the role of nutritional factors in cell growth, organ development and function, body composition, metabolism of nutrients in early life, and the physiology of normal and abnormal growth.

In human studies, special attention is being given to appropriate methods for assessing impaired intellectual and behavioral development and for evaluating the sociocultural environment of malnutrition--which in turn may influence mental development. Although emphasis has been placed on elucidation of the developmental consequences of severe maternal and child nutritional deprivation, possible adverse effects of the lesser degrees of malnutrition more commonly found in the United States are also being considered. The Branch continues to support a number of projects seeking to better define growth, body composition and nutrient requirements of normal individuals.

Definition of growth patterns, cellularity and metabolic processes of individual fat cells and adipose tissues in children and adolescents is the main thrust of several obesity studies funded by the Branch. Others are directed toward identification of the behavioral, genetic, endocrine and nutritional factors associated with overeating and weight reduction. Other studies seek to define the relationships of obesity with degenerative diseases and the effects on body composition of weight reduction through diet or exercise.

The Branch has implemented, through grants and contracts, a coordinated series of animal studies investigating the cellular and functional consequences of prenatal and postnatal malnutrition. Rat studies are seeking confirmation of preliminary data indicating that severe pre- and early postnatal malnutrition may influence subsequent nutrient utilization and the possibility that the consequences of malnutrition may be additive over generations. One grantee has recently reported that rats receiving diets marginal in lipotropic factors during gestation and lactation produced offspring appearing clinically normal at birth and postnatal development. However, the thymus and spleen of the deprived offspring were greatly reduced in size, as compared to controls, and could not respond normally to infections when challenged at early adulthood (three months of age).

The subhuman primate is utilized as a model for more direct correlation of malnutrition effects with those in man. One study is measuring the effects of closely quantified protein restriction of the rhesus monkey during pregnancy on prenatal development, growth and adaptive capacity of the infant. During the past year, preliminary data from this primate maternal nutrition study indicate that protein requirements during pregnancy may be considerably lower for the rhesus monkey than heretofore considered, provided that the diet is adequate in other nutrients. However, critically low levels of protein in the maternal diet has resulted in a 53% incidence of fetal mortality and reduced birth weight in infants of normal gestational age. Another researcher is seeking to differentiate the effects of early social deprivation accompanying protein restriction early in life on later social behavior of the rhesus monkey.

The impact of chronic moderate malnutrition during pregnancy on the development of the child continues to be the major area of emphasis in human studies. New data from the Guatemala study further substantiate earlier reports that maternal supplementation reduces the rates of prematurity, stillbirths, low birth weight and neonatal death. Furthermore, birth weight appears to correlate with the amount of supplementary calories consumed during pregnancy. Preliminary findings from this longitudinal study suggest differences in motor performance between those children born to women well-supplemented in pregnancy as compared to children from mothers who were not well-supplemented. A related study in rural Mexico is producing important results in the seriously neglected research area of maternal-infant interaction. Here, maternal supplementation during pregnancy has produced larger, more rapidly growing infants who also are physically more active and demanding of maternal attention. By the age of two years, supplemented children are six fold more active than unsupplemented counterparts.

This study further suggests that the smaller, less active, undernourished infant demands and gets less attention, leading to worsened nutritional status and to retarded behavioral development.

Future nutrition research directions of the Branch can be expected to include the areas of maternal/infant interaction, environmental influences on nutrition and development, better indices of nutritional status, latent adverse effects of fetal or early postnatal malnutrition, and better definition of nutritional requirements during adolescence.

Physical Growth

Physical growth research supported by the Growth and Development Branch encompasses developmental anatomy, cranio-facial growth, skeletal growth and studies in population genetics relating to anatomical development.

Projects supported in the area of developmental anatomy include descriptions of postnatal development of human cerebral cortex, lung growth, ultrastructural morphology of bone, as well as the growth and hypertrophy of various tissues or organs in health or disease. Cranio-facial growth studies are concerned primarily with identifying the genetic, metabolic and endocrine mechanisms involved and examining interrelationships of the different variables of facial growth and dentition. Several investigators seek methods for the eventual prediction of individual cranio-facial growth rates and patterns, and early diagnosis of abnormal cranio-facial development.

Skeletal growth studies include projects developing normative data on the bone weight, density and ash content of normal children and animals. Other projects involve longitudinal study of skeletal growth of low birth weight and normal birth control children, and children whose development may be compromised by severe malnutrition or chronic disease. Still other investigators, utilizing anthropometric data and radiographs from completed and ongoing longitudinal child growth and development studies, are seeking improved methods for evaluation of skeletal development, assessment of skeletal maturity, and the prediction of mature stature. During the past year, one investigator supported by the Branch has completed development of a set of growth tables which serve as norms for assessing growth during the first three years of life of low birth weight infants of varying gestational ages. Another investigator has, through development of a new parameterization of skeletal growth, achieved greatly improved reliability in predicting the mature stature of growing children.

Greater use of anthropometric measurements in assessing physiological status (particularly with reference to obesity and abnormal growth) is a promising research area. Future research support of the Branch may also be directed toward the determination of distribution of growth rates from birth through adolescence. These data would assist in better defining nutritional requirements and in determining the long-range implications of variations in growth velocity for health and performance.

Developmental Behavioral Biology

This section focuses on the biological mechanisms of behavior. Emphasis is placed on developmental studies, particularly, those that describe the effects of early experience on the developing brain and on subsequent behavior. The quantitative assessment of the maturation of sensory functions and perception in the developing infant and child and their modification by early experience is a major area of interest. Special attention is given to the measurement of anatomical, electrophysiological and chemical parameters of cerebral structure and function throughout the formative periods of development and consequent to early insults to the developing nervous system. Neonatal anoxia, exposure to toxins, specific nutrient deficiencies, and sensory deprivation are examples of the kinds of early insults and injuries that are being investigated.

Another item of programmatic concern is the clarification of neurobiological mechanisms that mediate abnormal social and emotional behavior consequent to "maternal-social" deprivation such as that experienced during the rearing of young primates in isolation. Behaviors commonly reported to follow such isolation rearing or parental deprivation (e.g., depression, social withdrawal, rocking, hyperactivity, hyperreactivity and violence) are critically relevant to the human condition and merit a high research priority.

Behavioral genetic studies are another area of programmatic emphasis. Genotypical characteristics of psychohormonal development (normal and abnormal) as well as genetics of special abilities, cognition, intelligence and achievement comprise the majority of studies in this area.

One grantee has conducted follow-up studies on the effects of artificial movement stimulation upon animals reared in isolation. In general, his experiments may be summarized as follows. Laboratory rearing with a moving artificial mother prevents the development of the stereotyped body-rocking characteristic of the mother-deprived rhesus monkey. Despite this, behavior of monkeys raised with moving or stationary surrogate mothers resembles that of other such monkeys more than that of wild-born monkeys. Thus, movement stimulation apart from other social variables does not eliminate all elements of the social deprivation syndrome. However, these results indicate that passive movement stimulation during rearing has abiding effects that go beyond the prevention of stereotyped rocking. On most tests, animals raised with moving surrogates resemble wild-born monkeys more closely than do animals raised with stationary devices. These studies have important implications for the nature of the sensory environment associated with child-rearing practices.

Another grantee has been studying sensory evoked responses of the brain in children and has related them to behavior. In a recently completed Mexican field study on malnourished marasmic infants (5-10 months of age), Barnett has found significantly longer latencies in the malnourished infants than normal controls. Preliminary analyses also show differences in amplitude, the number of components and morphology of the cortical evoked response. These studies highlight differences in the brain's processing of sensory information and offer unusual opportunities to assess the effects of malnutrition upon cerebral function and perception.

The many and varied issues of infant and child behavior can be neither clarified nor understood without knowledge of the cerebral processes that mediate such behavior. The development and nature of human brain function is singularly unique and central for understanding the excellence of human achievement and the tragedy of human failure. The technologies, instrumentation and research skills associated with brain research have reached an unprecedented level of sophistication which now permits a major commitment to the study of the developing brain and behavior.

Learning and Cognitive Development

This section of the Growth and Development Program has as its primary purpose the elucidation of the learning process, i.e., the way individuals acquire, extinguish, or modify knowledge, skills, and habits or behavior. It is recognized that learning and cognitive development, the process of knowing, are broad, integrative concepts. Efforts are now underway to discover how the human perceives, processes, and stores information in the brain. Some researchers confine their work to only one area at a time, others insist on studying the three aspects together. The major research areas included in this section are attention, perception, and the general psychomotor and sensory processes. The section also includes basic research which concerns conditioning, discrimination, memory, intelligence, the verbal and social aspects of learning, concept formation and reinforcement. While research concerning learning and cognitive development especially with infants and young children has been under the influence of stimulus-response theory, currently many devout environmentalists acknowledge the importance of inborn mechanisms of the infant which can provide a basis upon which behavior patterns will be formed. Since Piaget published in the mid 50's his account of spatial concepts, many developmental psychologists have been interested in a child's ability to perceive and to imagine spatial arrangements from different viewpoints. Illustrative of research supported by this section are two such experiments which were conducted to determine the relative contribution of age, sex, and three stimulus features (broad shape, object arrangement, and block shape) to perceptual accuracy on standardized adaptations of Piaget's three-mountain task. Significant differences in accuracy were obtained as a function of stimulus features, but features did not interact with age or with sex. Through this work with over 1,000 subjects the researcher is now studying the concept of egocentrism, the inability to take another's point of view. It would appear that egocentric error, as found in the three-mountain task, must be considered independent of the developmental construct of egocentrism. While significant strides are being made toward a better understanding of the learning process and cognitive development, we still are unable to designate the exact role of inheritance and the biological system versus the role of environment and experience upon human learning. Future efforts in this section will be directed toward bringing together scientists representing a variety of biological and behavioral sciences to help unravel this complicated process.

Human Communication Research

This section is concerned with the biological, psychological, and social factors and processes which are involved as children acquire and develop normal communicative skills. It is also concerned with those factors which

may interfere with normal acquisition and development of these skills as well as the role communication plays in human growth and development. While our interest includes all aspects of human communication, our current emphasis is on studies of the acquisition and development of such specific skills as speech, language and reading. It should be noted that many of the projects included in the Learning and Cognitive Development section, particularly those in verbal learning, perception, etc, are closely related to the area of Human Communication. Coordination of the activities and grant support in these two areas is, therefore, carefully maintained. A program project entitled, "Nature and Acquisition of the Speech Code and Reading" may serve to illustrate the type of research currently supported by this section. While this broad program of projects covers many aspects of speech and language, their recent work on reading deserves special note. It has been established that the principal difficulties most children have in learning to read are not with identification of the letter shapes as such, or even with the discovery that letters stand in an approximately one-to-one relationship to the sounds of speech, but in becoming sufficiently aware of the structure of spoken language to make mapping by an alphabet possible. Many young children are unable to explicitly analyze spoken language into syllables and especially into phonemes. The outcome of their most recent study shows that the encoded nature of the speech signal is central to the relationship between speech and learning to read. In the future the section will be anxious to obtain more data that will clarify the nature of the relationship between reading disability and failure in the ability to determine the number of phoneme constituents in spoken words. The section also will work toward a better understanding of the apparent predisposition man possesses for language acquisition and how this predisposition varies and is affected by non-genetic factors.

Personality and Social Development

The purpose of the personality and social development section is to program and administer research in the social and personality area of human growth and development. Current research is concerned with the development of exploratory behavior, ego development, intellectual development, and interpersonal attraction during infancy. Studies of childhood are examining the effects of race and integration on school performance as well as the relationships between early intellectual training and school performance. Other areas include children's achievement motives and the development of moral judgment. The section supports a few studies in later development, especially the effect of the college experience on adult role behavior and the effect of one's total life history, prior to college, on behavior. In addition to grants and contracts falling directly in the personality and social development area, this section also takes an active part in a variety of interdisciplinary studies, including a large contract program in nutrition and mental development.

One research program is trying to assess the contribution which contrasting patterns of child-rearing make to the development of social responsibility, activity, and individuation in the preschool years and early childhood, and to relate these data of early patterns of child-rearing and child behavior to types of identity formation achieved during early and late adolescence,

as well as to symptoms of dysfunction in childhood and adolescence. It aims specifically to examine the parent-child relationship found at Time 1 when the children were 4-5, at Time 2 when the children are 8-9 in order to determine the consistency of child and parent behavior, the interaction of parent and child behavior at Time 2, and the consistency of parent-child effects; to examine the correlates of sex-role typing for boys and girls; to relate the ethical phenotype to Kohlberg stage scores; to examine socialization antecedents and child correlates of creativity in children; to study the adaptation of the child in the school setting as a function of synchrony between patterns of school and home authority; to illuminate family interaction processes that are associated with children of different levels of competence, using videotaped data; to examine the interaction of functional vs. dysfunctional families; and to study cognitive aspects of development in middle childhood. This type of longitudinal study is very difficult to carry out but is very important for the kind of data the Growth and Development Branch is trying to develop.

ADOLESCENCE

Adolescence, a critical but little understood period of development, has been designated a priority area for research activities of the Growth and Development Branch. The Subcommittee on Adolescence Research of the National Advisory Child Health and Human Development Council has been active during this past year, meeting at frequent intervals and consulting with Branch staff members concerning adolescence research activities. In November, 1973, the Subcommittee presented to the Council a resolution, unanimously adopted by the entire Council, reaffirming support for a continuing emphasis on quality research on adolescent development and recommending several specific mechanisms for achieving this aim. These mechanisms include a proposed two-year program of small grants in adolescence research and the designation of training in adolescence research as a high priority need of the Institute.

All members of the staff have been involved in initiating the Expanded Research Program on Adolescent Development. A series of three multidisciplinary conferences have been held--"The Control of the Onset of Puberty," "The Nutrient Requirements of Adolescence," and "Adolescence in the Life Cycle." Each of these conferences will result in a major publication, with the first volume being released in April, 1974, and the other two currently in preparation. As a result of these conferences, the staff has identified the following five areas for major research emphasis:

1. Biological processes involved in the onset and completion of puberty.
2. Nutrition as it contributes to adolescent maturation and change.
3. Intellectual development during adolescence, including speech, language, and thought processes.
4. Adolescent socialization.
5. The relationships between hormones and psychological development during adolescence.

Growth and Development staff members have been involved in a variety of activities to encourage research in these areas. Widespread publicity for the expanded research program was achieved in August, 1973, when an announcement describing its goals was distributed to several hundred academic departments and research centers across the country. In addition, the announcement appeared in a number of relevant scientific journals. It is expected that we will be in receipt of a substantial number of quality research grant applications in the next months.

Besides corresponding and working directly with potential investigators, members of the staff have visited a number of leading adolescent clinics across the country. During these visits, information was gathered concerning the kinds of problems which appear most frequently among the adolescent populations treated, including those which the center personnel felt they understood and were equipped to treat, as well as those where they judged research is most urgent.

Several members of the Growth and Development staff serve on the Federal Interagency Panel for Research and Development on Adolescence, an organization which provides regular communication among all the federal agencies having major activities in the area of adolescence. In addition, contacts have been made with a variety of non-federal organizations concerned with adolescence. Staff members attend and participate in the meetings of the Society for Adolescent Medicine and serve as continuing members of the Youth, Health and Society Conference, an HEW-funded project to assess the nature of problems related to adolescent health care delivery and to find ways to improve the delivery of health services and education in the area of adolescent mental health. The diverse membership of this conference provides a channel of communication among federal agencies, non-federal agencies, universities, legislative offices, hospitals, private practitioners, and teenagers themselves.

Because adolescence research is basically a problem-oriented, rather than a discipline-oriented endeavor, it properly involves a number of scientific disciplines. The lack of multidisciplinary training opportunities in adolescence research is one factor which has impeded progress in this area. One of the goals of the Growth and Development Branch, therefore, is the establishment of research training opportunities in adolescence at institutions having access to appropriate study populations and the requisite interdisciplinary training expertise. Various mechanisms for achieving this goal are currently being explored.

For the future, the Growth and Development Branch envisions a continuing and major commitment to the encouragement and support of quality adolescence research. Besides the activities described above, members of the staff are engaged in the preparation of symposia on adolescence research for several professional organization meetings in the spring of 1974 and are planning a series of workshops on selected topics related to adolescence.

Recent research expenditures by NICHD in the area of adolescence have been modest. For example, in FY 1972, it is estimated that, exclusive of contracts, the entire amount of funds spent on adolescence-related research

(including research grants, training grants, fellowships, and RCDA's) represented 1.1% of the total NICHD expenditures. In FY 1973, this figure increased to 1.3%. Contracts in adolescence research for these two years totalled \$250,000 and \$211,000, respectively.

RESEARCH TRAINING ACTIVITIES

Beginning in January, 1973, the research training programs of the NIH, under the auspices of which the G&D Branch had supported the training of a substantial number of scientists, were phased out. While recent decisions have reversed this mandate for the immediate future, the Branch has welcomed the announcement of a new Research Manpower Development program and has been participating enthusiastically in the planning and programming of research training activities within this new program.

Three areas of research training are of primary concern to the G&D Branch at this time. The first is a multidisciplinary approach to adolescence research, which is discussed in the final portion of this section. The second area includes basic and clinical research training in a limited number of biomedical areas, as specified below. The third major area of Branch interest is for research training in the behavioral sciences, primarily in developmental psychology.

Within each of these last two major areas of research fellowship support there are specific subdisciplines which the G&D Branch designates as being of high priority. It should be emphasized that while the following areas are high priority, our research training needs are not limited to these areas.

A. Biomedical Research Fellowships

1. Fellowships in Developmental Pharmacology. Expansion of the number of scientists concerned with developmental pharmacology is needed to provide more basic research on the metabolism of medications, particularly those used in the treatment of children, with special attention to the influence of age-specific developmental periods on responses to pharmacologic agents.
2. Fellowships in Developmental Immunology. Body defense systems develop early in life and may be modified according to normal changes in physiological development or in response to insults such as malnutrition, which have their greatest impact at specific periods in time. It is essential that the surveillance system of the body is understood as it is involved in allergic and auto-immune diseases such as asthma, hay fever, Wiscott-Aldrich Syndrome and Lupus.
3. Fellowships in Human Genetics. Only a fraction of the total number of diseases and disorders which have been identified as being genetically caused are currently understood. Microtechniques, however, have advanced to the point where individual human genes can be isolated, chemically characterized, and even visualized under the electron microscope. Chemical

mapping of the entire human genome is no longer in the realm of speculation. Fewer than ten American geneticists are now working at the chemical level on normal human genetic material; many times this number are needed.

4. Fellowships in Developmental Neurochemistry. The field of neurochemistry is a relatively young one, having developed primarily within the past ten years. For years, research techniques were too crude and the brain too inaccessible to be well studied. Now, with the development of iontophoresis and microtechniques capable of recording the activity of a single neuron, the field of neurochemistry is on the verge of major breakthroughs.

B. Behavioral Sciences Research Fellowships

1. Fellowships in Developmental Neurobiology and Behavior. With increasing knowledge of the lasting effects of certain early experiences (such as malnutrition or maternal-social deprivation) on psychological development, it becomes increasingly important to specify precisely the central nervous system processes which accompany these behavioral effects. There is a need for fellowship support which would allow the well-trained developmental psychologist to attain specialized training in neurobiology, or for the neurobiologist to achieve sophistication in psychological methodology in order to advance this important interdisciplinary research area.

2. Fellowships in Socialization Processes. Training opportunities in this area would provide the needed interdisciplinary education and research experience for sociologists to further their training in psychology and for psychologists to increase their knowledge of sociology in order to do research on the interactions between developmental processes and social factors as they influence behavior throughout the life span of the individual.

3. Fellowships in Basic-Applied Social Research. One of the most pressing social-educational problems of our society is centered upon the large number of children who fail academically, do not learn to read, drop out of school, and fail to become contributing members of our society. Good research into the complex and interrelated causes of such failures is badly needed, but the traditional social science disciplines have too often isolated their students from these problems. This unique fellowship program in the basic-applied social sciences would provide an opportunity to introduce real-life situations to well-trained, research-oriented behavioral scientists.

C. Adolescence Research

The G&D Branch has made a commitment to encourage and support high quality research on important biological and behavioral aspects of adolescence. Strong encouragement for this expanded research effort has been provided by the National Advisory Child Health and Human Development Council, which has an active sub-committee on adolescence research.

There is a great need for more creative and methodologically rigorous researchers to investigate important aspects of adolescent development. This need may be met in part by training a new generation of scientists, prepared theoretically and substantively to apply new methods to the rapidly changing adolescent or to develop a new experimental model for human adolescence. Multidisciplinary research fellowship grants in adolescent development currently constitute our most pressing research training need.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 50640
Contract Title: Study of Relationship Between Nutrition and
Behavioral Development
Contractor: Pan American Health Organization, Institute of
Nutrition for Central America and Panama
Robert Klein, Ph.D.
Money Allocated: \$578,781; FY 1974

This contract has been designed to contribute additional knowledge of the relationship between nutritional status and physical, psychological, and social growth. Two pairs of small villages (aldeas) of approximately 650 inhabitants each have been matched on a variety of sociocultural variables. In one village of each pair a dietary supplementation program is provided with special effort made to include in this supplementation program every preschool child and pregnant woman in the village. Incaparina, a high protein food supplement, is made available to every person in the village who wants it. In the control villages medical service is offered, but no food supplementation is provided. Thus a sample of well-nourished preschool age children can be compared on relevant dimensions with a sample of medically treated preschool children raised under the dietary conditions characteristic of the area. Children will be followed from birth until about six years of age, at which time effects of differences in nutrition should be clear.

Four years of enrollment of pregnant women and their offspring were completed in Feb. 1973. This constitutes the basic population for long-term study of the effects of the supplementation program. It has been shown that birth weight is related to maternal height and weight at the onset of pregnancy: both are indicators of prior nutritional status. Days of illness during pregnancy also are directly related to decreased birth weight, apparently due to decreased food intake rather than infection per se. Most importantly maternal calorie intake is directly related to birth weight; maternal protein intake had little or no effect on birth weight but did appear to be related to infant morbidity, mortality, and growth. Pregnant women were supplemented for an additional year in these villages to increase the number of infants under one year of age to confirm these relationships. These data are now being analyzed. The data from the six month age period have all been completed. The results suggest differences in motor performance between the well supplemented and poorly supplemented children. Neurological and behavioral testing is continuing as the children mature in the study.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 81512
Contract Title: A Study of Malnutrition and Mental Development
in Bogota, Colombia
Contractor: Harvard University, Boston, Massachusetts
Frederick J. Stare, M.D.
Money Allocated: \$35,550 (U.S.-Japan Cooperative Medical Science
Program Funds); FY 1974

This project was designed to explore the impact of a food supplementation program on the development of pairs of siblings living in the slums of Bogota. One sibling was to be malnourished, the other not malnourished, thereby minimizing genetic and family differences between comparison groups. A number of new psychological test systems have been developed and demonstrated reliable. However, it has proven impossible to enroll sufficient pairs of siblings in the four cells of the study design. Therefore, the contractor is preparing the tests, findings, and conclusions from the pilot studies for publication. The contract was completed in January, 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Growth and Development Branch
Contract and Collaborative Research

Contract Number: NO1 12420
Contract Title: Research Materials and Assistance for Studies of Language
Development in Children
Contractor: Haskins Laboratories, New Haven, Connecticut
Franklin S. Cooper, Ph.D.
Money Allocated: \$74,100 (December 30, 1972 to June 30, 1974)

The original purpose of this contract was to provide specialized facilities and expert assistance to research scientists at other institutions (user groups) who are engaged in language studies relevant to children. In December of 1972 the contract was renewed for another 18 month period to continue to provide these services to user groups. In general, three kinds of assistance are provided to research groups, most of whom are working on projects of direct interest to the NICHD:

1. Research materials. Stimulus tapes for a wide variety of experiments constitute the primary direct assistance provided under this contract. Several methods of generating recordings suitable for research on spoken language are available including natural speech, synthetic speech, and non-speech sounds and noises prepared for presentation on either single-track or dual-track.
2. Specialized assistance. Professional and technical staff of Haskins Laboratories make available their knowledge and skill in the preparation of synthetic and natural speech recordings and in the use of their unique computer facility for generating precisely controlled binaural tapes.
3. Research training. A by-product of the visits by user groups has been a training experience in the status and methodology of basic speech research and an opportunity to gain some familiarity with the laboratory's entire program of research in speech which is supported in part by the NICHD.

During the initial 13 months of this contract, requests for assistance were received from 37 users affiliated with 23 leading universities. While it is difficult to characterize the projects for which users have requested assistance, they have in general clustered around a few types of problems: auditory discrimination in infants, language discrimination as a function of the child's age, and lateralization and dichotic effects as indicators of language processes. Only a few studies have been concerned with clinical problems, one on deafness and one on temporal lobe involvement.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Growth and Development Branch
Contract and Collaborative Research

Contract Number: NIH-NICHD 72-2735
Contract Title: Prediction of Mature Stature in Growing Children
Contractor: Fels Research Institute, Yellow Springs, Ohio
Alexander F. Roche, M.D.
Money Allocated: None in FY 1974

The objective of this two-year research contract, completed during FY 1974, was to develop an improved method for the prediction of mature stature. The prediction tables currently in use were developed 30-40 years ago, were based on small samples of children, and have not been validated. Under this contract, the investigator utilized radiographs and anthropometric data from the three largest longitudinal growth studies conducted in the United States to develop and validate a better parameterization of individual stature from birth through eighteen years.

The new method of predicting mature stature developed by co-investigators A. Roche, H. Wainer and D. Thissen, the "RWT" method, has now been demonstrated to be superior to alternative procedures, is based on data that are easily available in clinical practice, and can be applied throughout the entire period of growth. Previously, each different prediction method was applicable to children of a restricted age group. The "RWT" method for reliably predicting mature stature will greatly assist the clinical management of children of deviant stature (short or tall) and children receiving hormones or other therapeutic substances that can influence the rates of elongation and maturation of the skeleton. The predictions will also prove helpful in interpreting growth-related effects of cardiac and other surgery during childhood, and can assist in the selection of ages and sites for orthopedic procedures designed to equalize leg lengths.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Growth and Development Branch
Contract and Collaborative Research

Contract Number: NIH - NICHD 73-2069
Contract Title: Immunologic Responses of Normal and Malnourished Infants to Standard Antigens
Contractor: University of California, Los Angeles, California
Charlotte G. Neumann, M.D., M.P.H. and Richard Stiehm, M.D.
Money Allocated: \$94,700 (FY 1973)

The main objective of the project is to study humoral and cellular immune responses to standard antigens in normal and malnourished infants, with special emphasis on identifying changes due to nutritional deficiencies. The UCLA laboratories has developed methods for immunologic and nutritional assessment of malnourished populations in Ghana, Africa, which will be pre-tested on a small sample of North American children. Well nourished controls for the malnourished children will be selected on site in Ghana.

Four groups of subjects are studied:

1. Hospitalized Ghanian children with severe malnutrition
2. Ambulatory Ghanian children with moderate malnutrition
3. Age-matched Ghanian children without malnutrition
4. Age-matched U.S.A. children without malnutrition

The testing to be employed consists of:

1. A battery of immunologic testing including studies of deficiencies in antibody immunity, cellular immunity, granulocyte function, and opsonic function.
2. Assessment of nutritional status to include clinical nutritional assessment, anthropometric measurements, and biochemical assessment of nutrients.
3. Other studies to include routine hematologic studies, thick blood smears for malaria, stool exam for ova and parasites, and sickle cell preparations.

During the first contract year, data was obtained on 117 children but analyses are not yet complete. Information on adequacy of cell-mediated immunity and immunocompetence in malnourished versus normal children suggests the possibility of impairment, but must await collection of further data from adequate age-matched controls. The most intriguing finding has been the suggestion of decreased granulocyte bacteriocidal capability in malnourished infants, and further studies should reveal defects in phagocytosis or killing, whether intrinsic granulocyte functions or deficiencies in serum opsonins are involved. This contract will continue for an additional 5 months so as to get definitive data on the relationship between the nutritive state and the immune response in man.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Growth and Development Branch
Contract and Collaborative Research

Contract Number: NO1 22737
Contract Title: Analysis of Data from an Ecological Study of Infection, Malnutrition, and Growth of Children in a Guatemalan Indian Village
Contractor: Pan American Health Organization, Institute for Nutrition in Central America and Panama
Leonardo J. Mata, D.Sc.
Money Allocated: \$18,000 (U.S.-Japan Cooperative Medical Science Program Funds) FY 1973

The primary objective of this two year contract was the analysis and publication of the data from a longitudinal study of nutrition, infection and growth among pregnant and lactating women, infants, and young children in a small native village in Guatemala. Throughout the study attempts were made to interfere only minimally with the natural ecosystem of the village and family. Approximately 45-50 babies have been born per year in this village, providing a population in excess of 400 newborns over the eight year study period (1964-1971). Growth and morbidity data have been obtained on all children. More detailed studies on neonatal infections, their nature, and origin have been done on 350 children. Within this latter group, 45 (representing 50% of those born during 1965-1966) were identified for intensive study from the prenatal period through the first seven years of life. Regular data were obtained on these children concerning height, weight, days of illness, dietary intake and nutritional status, and infections and colonization of the intestine as indicated by weekly stool samples. Twelve of these had more intensive work-up of patterns of development of indigenous microflora.

During this contract, all of the data from the study have been entered into the computer at the University of Washington in Seattle and analyses have been completed. Dr. Mata, who is on sabbatical at the University of Washington, is now completing a monograph detailing the characteristics of the village, methods of investigation, and the extensive conclusions derived from detailed analysis of the data. It is anticipated that this monograph will be completed by summer 1974, and that it will be published in monograph form for the general scientific community. Following completion of the monograph, the principal investigator will continue analyses of these data to investigate questions such as birth weight and child growth as a function of parental height, weaning and child growth as a function of SES and fertility of mother, interactions between colonization with protozoa and the introduction of supplement, and growth as a function of increments of 150 and 300 grams in birth weight. This work is to be completed by spring 1975.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Growth and Development Branch
Contract and Collaborative Research

Contract Number: NIH-NICHD 71-2088
Contract Title : Non-Human Primate Colony
Contractor : University of California, Davis, California
Dr. Andrew Hendrickx
Money Allocated: \$253,000 (FY 1974)

Under this contract, a colony of rhesus monkeys (*Macaca mulatta*) of known medical, reproductive and geneological history is being developed at the California Primate Research Center, University of California at Davis. This colony serves as a resource of research animals of known quality from a standardized environment for investigators whose research is funded by the NICHD.

Animals of many stages of development including dated pregnancies, embryonic and fetal material, neonates of known gestation, mother-infant pairs, juveniles of known age, as well as certain biopsy material, tissues and fluids, are being made available to selected investigators, all of whom must be directly supported by NICHD. Application for animals is competitive and allocation is determined by an advisory committee which also advises on colony and contract management. Animals are shipped to recipient scientists throughout the country; a limited number of investigators can also be accommodated in visiting scientists' facilities on-site. Funds from the sale of animals are used to offset the costs of future contract years. Biological and behavioral data profiles are compiled and incorporated into the computerized record of each animal. This information is available to recipient investigators.

Demand for animals from this resource has reflected the rapidly increasing use of rhesus monkeys in recent years as an animal model for the human in biomedical research. Accordingly, a moderate expansion in colony size is being undertaken during the fourth contract year. New breeding females will be utilized primarily by investigators in studies requiring closely timed pregnancies.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01-HD-4-2816
Contract Title: Instrumentation Design for Electrophysiological Studies
Contractor: Research Institute, St. Joseph Hospital
David B. Coursin, M.D.
Money Allocated: \$90,000 (U.S.-Japan Cooperative Medical Science
Program Funds); FY 1974

The Research Institute, St. Joseph Hospital has a contract for the development of a portable electronic acquisition system that is capable of presenting a program of multiple sensory stimulation to subjects and recording their neurophysiological responses on magnetic tape. This technique will permit electrophysiological studies in the field with subsequent "off-line" sophisticated computer analysis of the taped data. This procedure is being developed particularly for use in the study of the effects of malnutrition on brain growth, development and behavior in human populations. However, it also will have extensive usefulness in evaluating neurophysiological consequences of a wide range of central nervous system disorders. It will be particularly valuable in providing a means of linking the biomedical with the behavioral aspects of a subject's evaluation.

The present contract began January 1, 1974, and has been active for four months. The portable electronic acquisition system is now in the operational prototype stage, capable of producing programmed pattern stimuli and recording two channels of electrophysiological data along with three event monitors. The workscope calls for further modification of the electronic system and expanding it to four channels of data recording. It also includes provisions for broadening the computer analysis program in collaboration with Dr. Bernard Saltzberg, at Tulane University, New Orleans. Finally, a series of simultaneous recordings of electrophysiological data will be made using the portable system and the regular research laboratory system at the Research Institute for comparison as to reproducibility, fidelity, and validation of the functional capability of the portable system.

At the present time, work is going forward on schedule in all of these areas. It is anticipated that within a year, decisions can be made regarding the possible wider application of the system through a group of collaborating nutrition research centers in various parts of the world.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01-HD-4-2859
Contract Title: Interactions between Malnutrition and Infection:
Antibacterial Defenses in the Newborn and Responses
to Measles Virus in Malnourished Children.
Contractor: Pan American Health Organization, Institute of
Nutrition for Central America and Panama.
Leonardo J. Mata, D.Sc., Michael Katz, M.D., and
Gérald T. Keusch, M.D.
Money Allocated: \$101,655 (U.S.-Japan Cooperative Medical Science
Program Funds), FY 1974

The two concurrent studies to be conducted under this new contract represent extensions of the long-term investigations carried out by Dr. Mata on the ecology of infection, malnutrition and growth of children in a Guatemalan Indian village. Samples and the data base supporting both studies will be provided by Dr. Mata's ongoing project.

In Project 1 (Dr. Keusch), the antibacterial defenses of the newborn will be evaluated by (a) assessing phagocytosis-associated metabolic activity of cord blood neutrophils, (b) measuring opsonic antibodies of cord sera, and (c) determining intracellular bactericidal activity of cord blood neutrophils.

In Project 2 (Dr. Katz), the ability of malnourished children to elicit and maintain appropriate humoral antibody response to measles vaccine and natural measles infection will be determined. An attempt will also be made to isolate wild measles virus(es) from this population as a means of considering relative agent/host factors responsible for the severity of measles in Guatemalan children.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Perinatal Biology and Infant Mortality Branch

The objective of the Perinatal Biology and Infant Mortality Branch is the promotion of a coordinated program of research and training which will enhance understanding and development of knowledge as related to pregnancy and maternal health, embryonic development, fetal growth, and infant well being through the first year of life. Efforts are also directed toward reducing this country's infant mortality rate, ameliorating infant morbidity, and narrowing the gap between the identification of new knowledge and its incorporation into the delivery of health care.

Program goals recognize the interrelationships of specific health and developmental problems encompassed in the prenatal, perinatal, and infant periods of life; and the effects that these events may have upon subsequent development and well being of the child. Particularly relevant are those morbidity- and mortality-related maternal health problems which effect fetal and infant health status, problems with which the newly born infant must cope in his adaptation to extrauterine life and subsequent survival and well being, and events occurring during the period of hospitalization following birth which can influence the subsequent behavior and development of the baby.

The Branch supports research and training concerned with various aspects of the physiology of pregnancy, disorders of pregnancy, placental function, immunologic phenomena, developmental biology, biochemical processes of embryonic and fetal growth, biophysical and biochemical antenatal and intrapartum diagnostic procedures, the process of labor and delivery, and genetic, pharmacologic, toxic, and infectious processes and influences which can effect the mother and fetus. Primary attention in the area of infant survival and well being emphasizes respiratory function, metabolic processes, thermal regulation, immunology and blood dyscrasias, infectious diseases, congenital malformations, and the sudden infant death syndrome.

Two major priority areas have been identified by the PBIM staff as a means of highlighting emphasis areas and guiding future program development. The first of these is to eradicate those maternal, fetal, and infant health problems which result in the highest incidence of infant morbidity and mortality. Chief among these problems are low birth weight, the sudden infant death syndrome, process of birth and extrauterine adaptation, abnormal fetal development, and complications of pregnancy.

The second major priority area is the identification of psychosocial factors in pregnancy and delivery which affect the fetus and outcome of pregnancy and contribute to infant morbidity and mortality. The emphasis in this priority area is on maternal stress, fears and conflicts and their influence on physiological functioning, postpartum maternal behavior, and maternal-infant relationships.

The Perinatal Biology and Infant Mortality Branch is organized into four broad program areas: Pregnancy and Maternal Health, Developmental Biology, Fetal Health and Development, and Infant Survival and Well Being. These program areas are under the supervision of two health scientist administrators and a pediatric medical officer who are responsible for the administration of research grants, training grants, career awards, fellowships, and contracts in each program area.

The Branch is responsible for the supervision and administration of approximately 21 percent of the Institute's extramural holdings, totalling about 322 grants and contracts and amounting to over 16.5 million dollars. The total program holdings, according to program sections, are shown in Table I. Approximately 8 percent of the grant holdings are concerned with training support. Eighteen of the training programs provide basic science research training in the field of developmental biology, two of which are closely allied to clinical departments and one is in a school of veterinary medicine. The remaining five programs are associated with clinical medicine and are conducted within departments of obstetrics and pediatrics. A serious gap continues in the PBIM training program in relation to the preparation of research neonatologists.

Areas of scientific responsibility and interest for each of the four program areas are highlighted in the following paragraphs; selected scientific accomplishments are included.

Pregnancy and Maternal Health

The Pregnancy and Maternal Health Section continues to support studies pertaining to normal and abnormal physiologic factors which influence the gravida state during the antepartum, intrapartum, and puerperium periods. Information is sought about the role of the cardiovascular system, respiratory system, endocrine glands, and genitourinary tract in pregnancy and their contribution and/or involvement in abnormal conditions during pregnancy. These include such conditions as toxemia, diabetes, hypertension, malnutrition, isoimmunization, viral and bacterial infections, blood dyscrasias, anemia and other hemorrhagic phenomena associated with pregnancy. The program is also concerned with placental function, factors involving the maintenance of pregnancy, the initiation of labor, the impact of common pollutants, drugs, and anesthetics on the mother and intrauterine conditions; and the psychosocial dynamics of pregnancy.

Scientific accomplishments achieved as a result of NICHD grant support are highlighted in the following paragraphs.

The effect of hormonal changes from estrogen to progesterone dominance on the contractile response of mammalian uterine muscle is an area of considerable interest. Dr. N. J. Naftalin and his research team at the Harvard Medical School studied the changes occurring in the isometric myogram of rat uterine strips obtained during the natural estrous cycle and pregnancy.

NICHD Grants and Contracts Active During April 1974
Perinatal Biology and Infant Mortality Program

Funds in thousands

| Mechanism of Support | Total | | Pregnancy and Maternal Health | | Developmental Biology | | Fetal Health and Development | | Infant Survival and Well-being | |
|----------------------|-------|----------|-------------------------------|---------|-----------------------|---------|------------------------------|---------|--------------------------------|---------|
| | No. | Amount | No. | Amount | No. | Amount | No. | Amount | No. | Amount |
| PBIM Total | 322 | \$16,534 | 85 | \$3,552 | 78 | \$4,658 | 81 | \$3,815 | 78 | \$4,509 |
| Research Grants | 252 | 12,631 | 75 | 3,377 | 58 | 3,302 | 66 | 3,125 | 53 | 2,827 |
| Training Grants | 23 | 1,663 | 2 | 31 | 14 | 1,233 | 3 | 150 | 4 | 249 |
| Fellowships | 11 | 138 | 4 | 41 | 1 | 15 | 3 | 42 | 3 | 40 |
| RCP Awards | 22 | 545 | 4 | 102 | 5 | 108 | 8 | 200 | 5 | 134 |
| Contracts | 14 | 1,557 | - | - | - | - | 1 | 299 | 13 | 1,258 |

Note: One research grant for \$49,108 paid from Special Genetics Funds of NIGMS is excluded. It is classified under "Infant Survival and Well-being."

These investigators demonstrated that a continuous estrogen influence is present in varying degrees throughout the reproductive life of the female mammal. Progesterone is present above a critical concentration. The major differences in the isometric myograms of the rates studied were the longer contractions and relaxation times of the estrogen-dominated muscles compared to those under progesterone influence. It has been found that as the onset of labor approaches, the progesterone block weakens and the pattern of myometrical activity alters towards that of estrogen domination. The longer contraction and relaxation times of the estrogen dominated muscle, together with the increased contractile potential of the pregnant muscles combine to provide the necessary cervix-dilating force required for efficient labor.

The origin of X-cells in human placental septa and basal plate has been questioned by numerous investigators. Several investigators believed that these mononuclear basophilic elements are maternal derivation. Dr. J. E. Waidman, and associates of the University of Illinois examined by quinacrine fluorescence of the Y-chromosome and presented chromosomal evidence of such cells to be trophoblastic and fetal origin. Y-body fluorescence in cells within placental septa and in the basal plate is observed in all male placentas examined. The frequency of Y-bodies in areas subsequently shown to be composed chiefly of X-cells occur in definitely trophoblastic areas. It was, therefore, concluded that most, if not all, placental X-cells are of fetal or trophoblastic origin.

Colonization with both Mycoplasma homines and with various strains of T-mycoplasmas was reported to be more prevalent among black than among white women and more prevalent among women who had never been married than those who had been married. Drs. J. S. Lin and E. H. Kass of Harvard Medical School developed a complement dependent mycoplasmacidal test for serotyping human genital T-mycoplasmas. These investigators indicated that T-mycoplasma isolated from the human genital tract are not serologically homogeneous. Sexual activity has been shown to be one of the important determinants of colonization with genital mycoplasmas. It is likely that several serotypes would be present in one individual. These investigators pointed out the need for caution in the epidemiological analysis of the distribution of genital mycoplasmas until the problem of heterogeneity has been studied.

It is widely believed that a hormonal stimulus is involved in the cause of the onset of labor and uterine contractions at/before term. Dr. M. E. Carsten of the University of California in Los Angeles demonstrated that the stimulus act on the contractile element of the uterus- the myometrium - through mobilization of intracellular calcium. In her studies, she isolated a sub-cellular tissue fraction from pregnant uteri. This fraction bound calcium in the presence of adenosine triphosphate (ATP) splitting ATP in the process. The preparations have been characterized morphologically and chemically by this investigator. The activity appears to be similar to ATP-dependent calcium-binding activity in sarcoplasmic reticulum isolated from striated muscles. The smooth muscle stimulator, prostaglandin E₂, inhibited ATP-dependent calcium binding and enhanced calcium release. It is suggested therefore that prostaglandins stimulate uterine contractility through transport of calcium.

Developmental Biology

The Developmental Biology section encourages strategic approaches to prenatal and neonatal health problems through support of fundamental research concerned with developmental events. Problems at organ, cell and molecular levels relating to low birth weight, prematurity, susceptibility to infant death and congenital malformations in the human infant are being pursued. Emphasis is on development at increasing levels of complexity in animal systems. Major areas of research include morphogenesis, pattern formation, developmental immunology, the genome in development, the organization of the cell, developmental endocrinology, and environmental impact on development. Investigators address fundamental questions to provide a broad research base for increased clinical acumen. Attention is directed both to investigations with mammals and to other model systems which afford distinct advantages for the study of development from conception through embryogenesis and into the first year of life.

Scientific accomplishments by investigators whose research is supported by the Developmental Biology program area follow.

Knowledge of the serum folate concentration during pregnancy is helpful in order to determine the etiology of an anemia. Current microbiologic assays for measuring serum folate concentrations, however, are time consuming and may be altered by antimicrobial and antineoplastic agents present in a blood sample. Dr. J. Caston of Case Western Reserve University recently described a direct competitive binding assay using tritiated pteromylomoglutamate, an unlabeled folic acid derivative, and a folate binder extracted from hog kidney. The binder, appears to bind the folic acid more tightly than previous compounds extracted from milk. The present assay is highly sensitive, rapid and, unlike microbiologic assays, is unaffected by antimicrobials and antineoplastic agents.

Although polypeptide chain termination is studied thoroughly in bacteria, only fragmentary evidence is available for the mechanism of the release of a complete protein in eukaryotic organisms including man. Dr. Joseph Ilan of Case Western Reserve University reports the isolation of a polypeptide release factor from the insect Tenebrio molitor. A cell-free, protein synthesizing system capable of polypeptide synthesis, but not capable of polypeptide release, was used. This system synthesizes predominantly one defined protein, namely cuticular protein. The released product was further characterized by chromatography. Information on the regulation of protein synthesis contributes to understanding of aberrant development in the human embryo.

Arylsulfatase is present in different forms in mammalian tissues. In man, a generalized deficiency of the enzyme is found in patients with metachromatic leukodystrophy, a progressive hereditary degenerative disease of the nervous system. The infantile type of disease is the most common form. Motor disturbance first appears between the first and second years of life and is followed by a gradual progression and degeneration. In the tissues of these patients, accumulation of undegraded products leads to degeneration of myelin.

Dr. Ronald G. Davidson and co-workers at the Children's Hospital of Buffalo have obtained, for the first time, visualization of the relatively low amount of arylsulfatase activity found in leukocytes, cultured skin fibroblasts and amniotic fluid cells. The high degree of sensitivity of the method permits the diagnosis of the enzyme deficiency prenatally. High-risk pregnancy can be monitored by the study of extracts of cultured amniotic fluid cells.

Fetal Health and Development

This program area is concerned with basic problems related to normal and abnormal development at tissue and organ levels. Research support continues to focus on areas pertaining to normal embryonic development and to specific environmental hazards or events of a pathophysiological nature detrimental to the growth of the fetus. Attention is given to nutritional, metabolic and other physiological sequences, immunologic factors, and pharmacological interrelationships between the mother and the developing fetus. Basic genetic, biophysical, and biochemical investigations are considered imperative, not only as a basis for assessing the fetal state, but also for meaningful antenatal diagnosis. As the placenta is the vital link between the mother and the fetus, investigations on the functional role of the placenta, particularly on feto-placental hemodynamics, feto-maternal gas exchange, and placental transfer of nutrients and drugs continue to be areas of interest. Selective research accomplishments for the past fiscal year are highlighted in the following paragraphs.

The normal fetal growth and development depends on adequate supply of oxygen. Many factors are important to the delivery of oxygen to the mitochondria of fetal tissue cells where oxygen is utilized. Drs. L. D. Longo, B. J. Koos, and G. G. Power of the Loma Linda University found that the amount of respiratory pigment, myoglobin, is a medium which facilitates the diffusion of oxygen and also serves as oxygen reservoir in the fetal muscle. They found that myoglobin concentration is about 50 percent of adult values in fetal heart, 20 percent in diaphragm, but is less than 1 percent in skeletal muscles. This probably reflects the relative activities of these muscles in utero. Evidence obtained so far indicates that myoglobin can as a short-term oxygen storage in the mitochondria of the fetal heart, and probably facilitates about 50 percent of oxygen diffusion in fetal cardiac muscle. Nevertheless these investigators believe, however, that myoglobin is probably not a significant factor in oxygenation of fetal muscle despite a relatively low fetal arterial oxygen tension.

Interrelationship between nutritional and genetic factors during pregnancy and their role in development of the offspring have been an area of high research priority. Dr. L. S. Hurley of the University of California demonstrated that dietary deficiencies of manganese can affect the genetic constitution of the animals studied. Normal development of otolith occurs when the diet contains 45 ppm manganese, the normal control level, but the response to low levels of

manganese in the diet varied considerably. The ability to store or release manganese for its utilization in pregnancy is thought to be under the control of specific genes. Observation of increased liter size with improved otolith development indicated that manganese deficiency produced metabolic effects other than shown by defective otoliths. These investigators pointed out that their findings suggest that at low or borderline levels of dietary intake of essential nutrients the responses of individuals may vary greatly depending in part on their genetic background.

Chromosomal studies relative to myeloproliferative disorders, blood disorders, and other diseases are receiving increased attention by many investigators as a basic approach for disease detection. Drs. D. H. Wurster-Hill, G. G. Cornwell, and O. R. McIntyre of Dartmouth Medical School made a careful chromosomal examination in one family. The results of this study revealed that four out of five siblings had chromosomal aberrations both in the myeloid and lymphoid cells. Karyotype shows frequently occurring cell aneuploidy and certain missing components. Trisomic cells from blood have additional chromosomes that occur in C group and other salient characteristics such as translocation of the long arms or without normal bands. Two of the siblings have had occupational exposure to organic solvents. Genetic predisposition to malignancy, chromosomal instability are possibly effected by environmental stresses.

It is known that neurological defects and mental retardation are prominent among children with D, (13-15) trisomy (mosaicism) in whom only a fraction of their cell population carries the chromosomal defect. Dr. M. Marin Padilla of Dartmouth Medical School made a detailed study of the fibrillar-neuronal organization of the cerebral motor cortex (area 4) of a newborn girl with (13-15) trisomy (Patau syndrome). A variety of structural abnormalities considered to be sufficient to explain, and possibly capable of causing some degree of cortical dysfunction, were uncovered in the motor cortex of this infant. A generalized hypocellularity was also found in the motor cortex in addition to a reduction in the number of neurons and glial cells. The possibility that the primordial cortical plate in this chromosomal defect may be primarily hypocellular was suggested.

Newborn calves receive all of their passive immunity through the colostrum. Calf fetus without such passive immunity is often subjected to many congenital infections that may lead to malformations and/or abortion. Dr. J. W. Kendrick of the University of California studied blood serums collected from bovine fetuses and newborn calves before they were given colostrum and also postcolostral calves. The values for immunoglobins G and M (IgG and IgM) were compared with the serum values for fetuses and precolostral calves with experimentally induced and naturally occurring congenital infection including those produced by chlamydia, Vibrio fetus, Coxiella burnetii, Anaplasma marginale, blue tongue virus, BVD and EBA. Specific antibodies

appeared in the calves infected with C burnetii, BVD, V fetus, blue tongue virus and chlamydia. Collection of serum samples from precolostral calves possibly having a congenital infection, utilization of the single radial immunodiffusion test, and specific serologic tests may provide a means of diagnosing intrauterine infections.

Infant Survival and Well Being

This program area is concerned with the postnatal period from birth to one year of age. Problems of low birth weight, the major single factor contributing to the high infant death rate in the United States, are emphasized. So also is the prevention of deaths that occur soon after birth in newborns in association with faulty adaptation to the extrauterine environment.

Basic and clinical studies of the etiology, pathophysiology, therapy and follow-up of conditions and syndromes such as asphyxia, respiratory distress, hypoglycemia, hyperbilirubinemia, anemia, erythroblastosis fetalis are supported. Equally important are the physiological, biochemical and behavioral aspects of extrauterine adaptation including respiratory, cardiovascular, thermodynamic, hematologic and metabolic changes associated with the crucial adjustments of the newborn at birth. Influences of maternal and environmental conditions and treatments affecting neonatal adaptation are studied to determine optimal nutritional, environmental, and metabolic requirements for both normal and high risk infants.

Of major interest are investigations on the effects of events and therapies during the early days of life on adaptation and subsequent development, particularly as they involve the high risk newborn and relate to such conditions as respiratory distress syndrome, acidosis, hypoglycemia, hyperbilirubinemia, phototherapy, and newborn infections. Research on etiology, pathophysiology, and management of congenital malformations during the newborn period is an important part of this program area. Findings resulting from grants supported through this section are highlighted in the following paragraphs.

Maternal and fetal infection during pregnancy is a major cause of fetal loss and morbidity. Asymptomatic maternal infections may cause severe and permanent fetal damage. Some infections occurring at different stages of gestation cause specific fetal and neonatal disease processes. Dr. Charles A. Alford of the University of Alabama has continued studies on women with chronic intrauterine infections and has followed their infants in longitudinal development. Progress has been made in defining the significance of maternal seroconversion to various viral infections during pregnancy. The role of cervical infection with herpes virus and subsequent transmission to the neonate has demonstrated differences in herpetic strain virulence. Other studies have been directed at cytomegalovirus infection and its detection and treatment in the asymptomatic gravid female and her infant. Dr. Richard Stjehm of the University of California in Los Angeles has continued to study deficiencies of phagocytic and opsonic function in the blood of newborn infants. Of significance has been their observations that alpha -1- fetoprotein IgG, and albumin can serve as a useful index of gestational age in the

human infant. Dr. Bascom Anthony of Harbor General Hospital in Torrance, California has initiated studies to test various mechanisms of recognizing and treating the group B streptococci. This group of organisms has recently emerged as the most frequent causative agent of fatal neonatal sepsis and neonatal meningitis.

Pulmonary maturation and the synthesis of dipalmitidyl lecithin by the fetal and neonatal lung continues to merit considerable scientific inquiry. Dr. George Brumley of Duke University in Durham, North Carolina has continued studies to elucidate the pathways of lecithin synthesis and the relationship of intrauterine asphyxia to neonatal pulmonary development. Dr. Peter Auld of Cornell University in New York City has concentrated efforts on the functional physiology of the immature lung and how the functional integrity of the lung changes with development during the respiratory distress syndrome and with variations in the composition of pulmonary surfactant.

At the University of Minnesota, Dr. John W. Reynolds has been investigating postnatal adrenal cortical function in premature infants with respiratory distress syndrome of varying degrees of severity. By measuring serum cortisol levels in these infants, Dr. Reynolds has not observed associated postnatal adrenal hyposecretion in infants with fatal hyaline membrane disease. His data questions the mechanism cited by recent findings of acceleration of pulmonary maturation after antenatal maternal administration of steroids.

As the newborn infant adapts to his new and changing environment, his ability to maintain a normal blood pH is constantly challenged. Of value to clinicians in the recognition and treatment of disturbances of acid-base equilibrium are the investigations being done by Dr. R. W. Winters at Columbia University. His studies included quantitation of acid-base displacement in small premature infants and infants with chronic diarrhea. By a detailed evaluation of computer-processed data, these researchers have established confidence bands for metabolic acidosis, chronic respiratory acidosis, and metabolic alkalosis. These standards are useful to the clinician in determining the acid-base status of the sick newborn. Dr. Winters has also embarked upon a large comparative study on the metabolic effects of systematic variation in protein intake in newborn infants receiving total parenteral nutrition. Nitrogen and fatty acid balance will also be closely studied. The usefulness of plasma and urine RNase in infants receiving total parenteral nutrition is being assessed as an adequate indicator of nutritional adequacy in human neonates and growing infants.

Sudden Infant Death Syndrome

During the past year, the Perinatal Biology and Infant Mortality Branch has continued its efforts to expand the Institute's sudden infant death syndrome program. This syndrome, also referred to as crib death, is a worldwide public health problem. It is the leading cause of death in infants between the ages of one month and one year. The incidence rate is between 2.0 and 3.0 per 1,000 live births, accounting for the deaths of 7,500 to 10,000 apparently healthy infants each year. Victims are predominantly between the ages of one and six months; they die suddenly, quietly, and unexpectedly in their cribs. In the

majority of cases, the baby is apparently in good health. He does not have a cold or infection and takes his feeding without difficulty. The infant is then placed in his crib for a nap or for the night; several hours later, he is found dead. At autopsy no significant cause of death can be found.

The Institute's sudden infant death syndrome program continues to be a broadly based operation of research to further increase our understanding of underlying mechanisms of the syndrome, to discover its probable cause(s), to identify infants at risk of becoming its victims, to explore preventive approaches, to inform the scientific and lay communities about the sudden infant death syndrome, and to stimulate scientists to direct their investigative efforts toward finding the solution to this complex biomedical problem. Other objectives of the SIDS program include developing guidelines for use by coroners, medical examiners, and pathologists in handling these cases, support for interdisciplinary conferences and workshops concerned with the sudden infant death syndrome, and the preparation and distribution of scientific and public information documents.

The Institute's seven emphasis areas for SIDS research continue to receive major emphasis. These include:

1. Developmental neurophysiology as it may relate to the sudden infant death syndrome, with particular emphasis on abnormal sleep patterns as associated with autonomic disturbance which may interfere with vital functions.
2. Respiratory-cardiovascular responses to stimuli, such as hypercapnia and hypoxia, and unusual vagal effects in relation to susceptibility to the sudden infant death syndrome.
3. Problems related to the developmental aspects of heat production, thermal regulation and relevant ambient conditions which may be associated with the sudden infant death syndrome.
4. Abnormal perinatal development of immune response mechanisms, with emphasis on the relationship of deficits in immunologic competence as a predisposing factor in the occurrence of the sudden infant death syndrome.
5. Psychological stresses experienced by parents, other relatives, and the community in association with the occurrence of a sudden and unexpected infant death.
6. Epidemiology of the sudden infant death syndrome.
7. Anatomic pathology of the sudden infant death syndrome.

During FY 74, the Branch sponsored three research planning workshops and conferences relevant to these seven priority areas. These included: Recognition of Infants at Risk: An Approach to Prevention; Development of Upper Respiratory Form and Function; and Post Mortem Evaluation for Investigative Efforts.

Each of these workshops brought together groups of scientists knowledgeable in the specific areas of the subject matter to be discussed, but not necessarily familiar with the phenomenon of the sudden infant death syndrome. The purpose of these workshops was to consider the problem of crib death, to identify new approaches to investigating the syndrome, as related to the specific emphasis area and to highlight specific research questions in need of in-depth study. A summary report for each workshop is being prepared for publication.

The results of the SIDS research planning workshops are being used as a base for expansion of the Institute's SIDS research grant and contract program. During FY 74 significant new leads have emerged from these workshops and NICHD supported research pertaining to SIDS. For example,

1. SIDS IS RELATED TO SLEEP DEPRIVATION

Dr. Dennis McGinty, Department of Psychology, University of California School of Medicine in Los Angeles, has studied the relationship of apnea to sleep patterns in kittens. It was observed that spontaneous apneic periods occurred during both slow wave sleep (SWS) and rapid eye movement sleep (REM) states. These episodes of apnea were usually, but not always, associated with arousal. Arousal during apnea was less frequent during REM and following sleep deprivation. Furthermore, the frequency of apnea was greatly increased following sleep deprivation. Moreover, spontaneous laryngeal spasms occurring during REM sleep were also observed. These observations are significant because a prolonged apneic period occurring at a time when arousal mechanisms are suppressed could lead to anoxia and central nervous system depression. Such depression could result in sudden death without pathologic findings. It is possible also that a phasic laryngeal spasm occurring at a time when arousal mechanisms are depressed could precipitate irreversible respiratory failure.

2. SIDS IS CHARACTERIZED BY CHRONIC SLEEP RELATED HYPOXIC EPISODES

Dr. Richard Naeye, Department of Pathology, Hershey Medical Center, Pennsylvania State University has demonstrated hypertrophy and hyperplasia of smooth muscle fibers in small pulmonary arteries of SIDS babies. The findings suggest that these infants suffered chronic alveolar hypoxia since such hypoxia is associated with increased pulmonary vascular smooth muscle mass.

3. SIDS IS RELATED TO IMMATURETY OF THE NERVOUS MECHANISM CONTROLLING HEART FUNCTION

Dr. Edward Hon, Department of Obstetrics, University of Southern California, has for the first time recorded detailed biophysical and biochemical measurements in a human fetus and newborn that subsequently was a victim of SIDS. This SIDS victim showed a blood pH of 7.1 at

birth (normal pH - 7.35 - 7.40) and 7.16 after eight minutes of life. His fetal heart rate (FHR) tracing at 37 weeks gestation resembled, at times, the FHR of an infant at 28 weeks gestation. There may be a relationship between susceptibility to SIDS and evidence of immaturity in the autonomic mechanisms controlling fetal heart function; a tendency toward acidosis in the intrapartum and immediate post partum period; and greater than usual variation in systolic time intervals (electrical-mechanical relationship) in the newborn period. It may be that immaturity of the nervous mechanism controlling the heart function can be identified in the fetal heart rate during the process of labor.

4. APNEA IS A DEVELOPMENTAL PHENOMENON AND SIDS IS RELATED TO HEART RATE CHANGE INDUCED BY SLEEP APNEA

Dr. Alfred Steinschneider, Department of Pediatrics, Upstate University of New York at Syracuse, will test his hypothesis by studying the frequency of apnea, the interrelationship between apnea and cardiac rate, and the influence of state of sleep on the incidence of apnea in infants weighing less than 2,500 grams at birth.

5. SIDS MAY BE ASSOCIATED WITH PULMONARY VASCULAR OBSTRUCTION DUE TO HEMAGGLUTINATION AND HEMADSORPTION

Dr. James Mason, Department of Pathology, University of Tennessee will test this hypothesis by performing viral cultures on autopsied SIDS victims, as well as immunofluorescent and electron microscope studies in viral localization. Determination of pO_2 concentrations in the left ventricular blood from these victims will permit this investigator to determine whether the babies died primarily from respiratory failure. Individuals with pulmonary death tend to have a low pO_2 in the left ventricular cavity for many hours after death while patients who die from nonrespiratory causes have higher pO_2 levels in the left ventricle.

6. SIDS RESULTS FROM APNEA SECONDARY TO AN INADEQUATE RESPONSE TO SUDDEN RESPIRATORY LOADS

Dr. Heather M. Bryan, Hospital for Sick Children, Toronto, Canada, hypothesizes that respiratory load compensating mechanisms are underdeveloped in infancy and that this contributes to the occurrence of SIDS. Adults are able to respond to increased respiratory loads by increases in tidal volume because the effective elastance of their respiratory system is far greater than the passive elastance of the same system. This response, in turn, affords ventilatory stability. Conversely, because the passive elastance of the respiratory system is reduced in infants, their "effective elastance" and ventilatory stability also may be reduced. Moreover, since the Hering-Breuer reflex is more active in infants than adults, respiratory loading resulting in decreasing tidal volume might also increase inspiratory duration and decrease frequency in infants.

7. SIDS MAY BE RELATED TO ADULT LIKE APNEA SYNDROMES

Dr. William Dement at Stanford University is studying the relationship between infants who have "stopped breathing" and the occurrence of sudden infant death syndrome. This study seeks to identify a possible relationship between respiratory abnormalities and sleep. Respiratory abnormalities such as obstructive and diaphragmatic apnea will be studied. The respiratory abnormalities will be correlated with secondary cardiac changes and measurements by oxymetry of oxygen saturation in the blood. A second component of this investigator's study seeks to define a population "at risk" for sudden infant death or sleep apnea by studying families in which SIDS has occurred and in families in which sleep apnea syndrome has been studied.

8. SIDS RESULTS FROM DEPRESSION OF CHEMOSENSITIVE REFLEXES DURING SLEEP

Dr. Alfred Krauss of Cornell Medical Center is studying respiratory chemosensitivity during sleep in infancy. By testing responses to various levels of carbon dioxide while monitoring minute volume ventilation during sleep and in the awake state, Dr. Krauss seeks to identify whether depression of chemosensitivity takes place during sleep in infancy.

9. SIDS IS RELATED TO MALFUNCTIONING AUTONOMIC REFLEXES

Dr. Peter Katona of Case Western Reserve University seeks to demonstrate a link between the sudden infant death syndrome and malfunctioning autonomic reflexes. In this study, Dr. Katona is characterizing normal development of autonomic reflexes by serial studies of infants to test the hypothesis that autonomic reflexes undergo a period of hypersensitivity or instability during the period of development (2-4 months) when SIDS occurs most frequently.

10. SLEEP MATURATION AND SIDS

Dr. Robert Mellins of Columbia University Babies Hospital is also assessing the effect of maturation and sleep on control of respiration in full term and premature infants in a longitudinal follow-up. Patients are also tested for their ventilatory response to inhaled carbon dioxide while sleeping and awake.

The Institute has provided support to the Division of Laryngology and Otolaryngology of the Johns Hopkins University, School of Medicine to explore crying patterns of an infant dying of sudden infant death syndrome as compared to non-SIDS infants. The Institute has also supported the reporting of state-behavior analysis of another infant who died of SIDS.

The branch has also prepared and announced two Requests for Proposals:

1. Evaluation and follow-up of selected respiratory, cardiac and neurophysiologic parameters in infants who are reported to "have stopped breathing."

2. Development of the structure and function of the upper respiratory tract.

Knowledge obtained from these studies will be of vital importance in understanding basic physiological mechanisms involved in SIDS, as well as, more completely understanding the morphological development of the nose, mouth, pharynx, and larynx in the human newborn.

Congressional hearings on the sudden infant death syndrome were held on August 2, 1973 for HR 9585 and September 22, 1974 for S. 1745. Program staff prepared testimony and served as support staff at the hearings. On April 22, 1974, President Nixon signed the Sudden Infant Death Syndrome Act of 1974 (P.L. 93-270). This Act:

- o fixes by statute the responsibility of the National Institute of Child Health and Human Development (NICHD) for the conduct of SIDS research;
- o requires the Secretary to carry out a program to develop public information and professional educational materials relating to sudden infant death syndrome (SIDS);
- o authorizes the Secretary to make grants and enter into contracts for projects to collect, analyze, and furnish information as to the causes of SIDS and to provide information and counseling to families affected by SIDS; and authorizes for those grants and contracts appropriations of \$2 million, \$3 million, and \$4 million for fiscal years 1975, 1976 and 1977 respectively;
- o requires various reports to the Congress or its Committees as to SIDS research and projects and on internal budgetary requests.

In October 1973, the PBIM convened a meeting of representatives of DHEW programs involved in SIDS related activities in order to enhance communication between the programs as to the extent of the Federal effort in SIDS. In January 1974, at the request of Assistant Secretary Edwards, this group formed the nucleus of the DHEW Interagency Panel on SIDS. The panel has representation from the Assistant Secretary's office; National Institute of Child Health and Human Development; National Institute of Neurological Diseases and Stroke; National Center for Health Statistics; National Institute of Mental Health; and Maternal Child Health Program-Bureau of Community Health Services. Its purpose is to coordinate Federal activities in SIDS and foster communication between the groups. The PBIM Program Director, Dr. Hasselmeyer, and the Assistant Director, Biological Sciences, OPPE, NICHD, Mr. Hunter, serve as co-chairpersons. Three meetings have been held between January and June 1974.

Program staff have continued to work with the National Center for Health Statistics for the inclusion of a specific category for sudden infant death syndrome in the ninth edition of the International Classification of Diseases.

During FY 74 the program has sponsored a lecture series entitled "New Research Perspectives in SIDS-1974" which brings to the professional and lay communities new research advances by NIH supported investigators. There have been two lectures in the Bethesda area. Dr. Alfred Steinschneider of Syracuse University presented the first lecture on "Recognition of Infants at Risk for SIDS" on February 19, 1974, and Dr. Richard Naeye of M.S. Hershey Medical Center, Pennsylvania State University spoke on "Hypoventilation: A Possible Cause of SIDS" on April 5, 1974. Additional lectures are planned for the NIH campus, as well as at sites across the nation.

Program staff has sought to collect and update existing bibliographies on SIDS and other relevant aspects with the National Library of Medicine. Two bibliographies are in press regarding "Parental Response to Infant Death," and "Respiratory Control Development." In addition program staff have prepared several articles for professional journals regarding research developments in SIDS.

Program staff have participated in many formal discussions, seminars, and symposia regarding SIDS program activities. Lectures and formal presentations have been made by staff to physicians, medical students, nurses, and parent organizations. Dr. Hasselmeyer and Mr. Hunter gave the keynote address at the National Foundation for Sudden Infant Death Fourth Annual Meeting of Chapter Presidents. They also conducted grand rounds on SIDS at the Children's Hospital in Honolulu. Dr. Hasselmeyer addressed the PHS Commissioned Officers Association Annual Meeting and will keynote the 1974 International Conference on Causes of Sudden Death in Infancy being held in May in Toronto, Canada. Dr. Hasselmeyer was the guest speaker at the Howard County, Maryland Chapter and the Bowie, Maryland Chapter of the Guild for Infant Survival and the Bowie Nurse's Association. Dr. Merritt, PBIM Pediatric Medical Officer spoke before the Kansas Medical Society in Wichita on new research developments in SIDS, and addressed the Wichita Chapter of the National Foundation for Sudden Infant Death. He also served as a panel member on a SIDS program sponsored by the District of Columbia's Human Resources Maternal and Child Health Agency. He discussed the Federal effort in the sudden infant death syndrome.

Conferences

In addition to the previously mentioned SIDS conferences and workshops, the program sponsored an interdisciplinary conference in April 1974 entitled Phototherapy for Hyperbilirubinemia: Long Term Implications. At this conference the state of the art, possible detrimental and long-term developmental effects of phototherapy and research needs were emphasized. In addition, possible detrimental sequelae were sought in data from long-term clinical experience. Physical science approaches to dosimetry were also explored.

An interdisciplinary conference entitled Perinatal Intensive Care will be held in June 1974. Obstetricians, neonatologists, nurses, and engineers will consider new approaches to investigative work in light of the current state of knowledge concerning the impact of intensive care upon infant survival and well being.

The previously mentioned SIDS conference concerned with development of the upper respiratory structure and function was a direct outgrowth of the SIDS planning workshops on cardiorespiratory phenomena. It was co-sponsored by the National Institute of Child Health and Human Development, the National Institute of Dental Research, and the John F. Fogarty International Center.

The National Institute of Child Health and Human Development and the National Heart and Lung Institute are both interested in lung maturation, neonatal respiratory adaptation, and neonatal respiratory distress syndrome. Recent reports in the literature indicate that lung maturation may be enhanced by the administration of cortico-steroids to the pregnant woman in the last weeks of pregnancy. If this is so, it might be possible to induce lung maturation in fetuses where premature birth is threatened, and concurrently prevent the development of RDS and related mortality. In conjunction with this common interest, the NICHD and NHLI co-sponsored a workshop on Possible Pharmacological Mechanisms for Enhancement of Lung Maturation for Prevention or Treatment of Neonatal Respiratory Distress. A report of the workshop is being prepared.

The Branch also provided partial support for the Perinatal Research Society's annual interdisciplinary conference.

Publications

The program staff prepared for presentation to the National Advisory Child Health and Human Development Council on November 26, 1973 a document entitled Perinatal Biology and Infant Mortality Program: Objectives and Priorities.

The following publications have also been published in this fiscal year:

Longo, L. D. and Bartels, H., Respiratory Gas Exchange and Blood Flow in the Placenta. DHEW Publication No. (NIH) 73-361, 1973. pp. 570.

Shepard, T. H., Catalog of Teratogenic Agents. Baltimore: The Johns Hopkins University Press, 1973. pp. 221.

Kretchmer, N. and Hasselmeyer, E. G., Horizons in Perinatal Research: Implications for Clinical Care. New York: John Wiley and Sons. 1974, pp. 193.

Shereshefsky, P. M. and Yarrow, L. J., Psychological Aspects of a First Pregnancy and Early Postnatal Adaptation. New York: Raven Press, 1974, pp. 373.

Niu, M. C. and Segell, S. J., The Role of RNA in Reproduction and Development. New York: American Elsevier Publishing Co., 1974, pp. 357 (Supported through grant funds).

Dancis, J., and Hwang, J. C., Problems and Priorities in Perinatal Pharmacology. New York: Raven Press, 1974.

RESEARCH PLANNING WORKSHOPS ON THE SUDDEN INFANT DEATH SYNDROME:

- Hasselmeier, E. G. and Knox, G. E., 1. Behavioral Considerations. DHEW Publication No. (NIH) 74-577.
- Dancis, J., 2. Developmental Aspects of Infection and Immunity. DHEW Publication No. (NIH) 74-578.
- Segal, S., 3. Cardiorespiratory Phenomena. DHEW Publication No. (NIH) 74-579.
- Weitzman, S. D., and Graziani, L., 4. Neurophysiological Factors. DHEW Publication No: (NIH) 74-580.
- Stark, C. R., and Froggett, P., 5. Epidemiology. DHEW Publication No. (NIH) 74-581.
- Beckwith, J. Bruce, 6. Pathology. DHEW Publication No: (NIH) 74-582.

There are three additional SIDS publications that are in the process of publication. These include one booklet of information for the rescue worker and parents, one booklet concerning an overview of SIDS for the lay public, and one booklet reporting the results of an Institute supported survey of management of SIDS cases.

Foreign Country Activities

PL - 480 Program: The PBIM Program administers two PL - 480 research projects in Yugoslavia (Dr. S. Milkovic and M. Skreb) and one in India (Dr. S. K. Arya). Dr. Skreb visited NIH and research laboratories of the U.S. Project Officer in California in 1973 as part of the collaborative efforts. Two proposals from Poland and one from Pakistan are under review. PBIM will be participating in a new U. S. - Polish collaborative research program which is in the planning stages.

INSERM: The PBIM Program has been participating in the NIH collaborative research activities and exchange program with the Institute National de la Sante et de la Recherche Medicale (INSERM) in the area of perinatology under the coordination of the Fogarty International Center. The United States coordinator for this area is Dr. Norman Kretchmer, Professor of Pediatrics and Human Development at Stanford University.

Personnel

The PBIM Program is assigned four professional positions; one Secretary-Grants Assistant; one clerk-stenographer; one clerk-typist. Three of the professional positions are staffed with persons holding Ph.D. degrees. One of the professional positions is staffed by a pediatric medical officer. The program is also assisted on a part-time basis by a professional staff person assigned to the Planning and Evaluation Program who serves as Executive Secretary for PBIM research contracts and assists in handling other program activities.

In FY 73; one NIH Grants Associate worked in the Branch for seven weeks during which time he evaluated the PBIM program in SIDS in relation to its being on Operational Planning System in FY 1972.

The Branch has actively participated in the NIH Upward Morbidity Program. One stay-in-school student has worked in the program for the past two years. One secretary is currently enrolled in the NIH Federal City College Program.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : Intrapartum Fetal Monitoring

Contractor : Professional Staff Association of the Los Angeles County/
University of Southern California Medical Center,
Los Angeles, California

Money Allocated : FY74 \$164,600 - 12 months

Objectives: The objective of this project is to characterize the changes and patterns associated with specific groups of high-risk obstetrical patients through detailed study and analyses of fetal heart rates (FHR), fetal electrocardiograms (FECG), uterine contractions (UC), and fetal acid-base status and respiratory gases. This work should help to clarify the value and merits of biochemical and biophysical methods of fetal surveillance in specific groups of high-risk obstetrical conditions. The high-risk obstetrical conditions to be studied include toxemia, chronic hypertension, Rh sensitization, abruptio placentae, maternal diabetes, premature rupture of membranes, premature labor, prolonged pregnancy, para 6 or greater, elderly primigravida, breech presentation, previous cesarean section and maternal hyperpyrexia.

Significance to Biomedical Research and Program of the Institute: As the result of applying higher standards of medical care to obstetrics during the past thirty years, maternal mortality has decreased tenfold. Over the same period, perinatal mortality has remained relatively unchanged even though similar high standards of medical care have been employed. In 1964 in the United States, from a total of about 3.7 million births a year, there were 28 perinatal deaths per 1,000 live births; the perinatal death rate in 1967 was 26.2 per 1,000 live births. This number of deaths is matched by a similar amount of perinatal morbidity which includes respiratory distress syndrome, mental retardation, congenital heart disease, and other malformations.

Since a large proportion of perinatal mortality is associated with the last week of gestation, it is pertinent to examine current methods of checking fetal condition during the course of labor. During the last decade, biochemical and biophysical techniques, separately and in combination, have been proposed as methods of fetal surveillance during labor and delivery. Since a sizable proportion of perinatal morbidity and mortality may be directly related to the stresses of labor and delivery, it is important that the relative merits of these fetal monitoring techniques for fetal and neonatal survival and well being be determined, particularly in relation to specific high-risk obstetrical conditions.

The complementary use of biochemical and biophysical methods to study the fetus, during labor and delivery, and the infant through the period of extrauterine adaptation provides valuable information not previously available. Information obtained will aid in delineating the respective value of biochemical and biophysical techniques for fetal surveillance in specific types of high-risk pregnancies.

Proposed Course: Subjects are selected from "high-risk" patients who have diagnoses including toxemia, chronic hypertension, Rh sensitization, abruptio placenta, maternal diabetes, premature rupture of the membranes, premature labor, prolonged pregnancy, para 6 or greater, elderly primagravida, breech presentation, previous cesarean section, and maternal hyperpyrexia. High-risk patients are viewed from the standpoint of the Goodwin high-risk score and the specific high-risk indication, e.g., toxemia, and compared with patients without complicating obstetrical conditions.

Basic biophysical (FHR, FECG, UC) and biochemical (pH, pO_2 , pCO_2) data are collected from detailed study of the mother and fetus during labor and from the newborn through the first hour of life. Maternal and neonatal conditions are assessed at 24 and 48 hours following delivery.

As of December 30, 1973, 504 patients were monitored; this completed sample acquisition.

Electronic data processing facilities are used to handle data accruing from these investigations. Data are being subjected to descriptive and statistical analyses (correlations, Chi-squares and multiple regression). A detailed profile is being developed on each group of high-risk indications. In this way it will be possible to consider the group as a whole or to compare one indication with another.

A clinical index of fetal risk is also being developed. This index takes into consideration the clinical maternal risk factors, baseline fetal heart rate, abnormal fetal heart rate patterns, acid base status of the fetus, and uterine activity.

It is anticipated that this project will be completed by December 1974.

NICHD Annual Report

July 1, 1973 through June 30, 1974
Perinatal Biology and Infant Mortality Branch

Contract and Collaborative Research

Contract Title: Proposed Analysis of a Major Cause of the Sudden Infant Death Syndrome (SIDS).

Contractor: Milton S. Hershey Medical Center
Pennsylvania State University
Hershey, Pennsylvania

Money Allocated: FY 74 \$49,800 - 19 months

Objectives: The purpose of this study is to investigate five post-mortem parameters that reflect ante-mortem chronic alveolar hypoxia (including apneic spells). The right ventricle of the heart, small pulmonary arteries, carotid body, bone marrow, and renal glomeruli will be studied by qualitative and quantitative neurohistology. The glial fibrils, astrocytes, astroglia, microglia, and oligodendroglia will be studied in areas known to be respiratory centers. Furthermore, neural synapses in the reticulo-spinal tract will be quantified. Victims of sudden infant death syndrome and age matched controls of other infants dying from unrelated causes will be used.

Significance to Biomedical Research and Program of the Institute: Previously sudden infant death syndrome was a diagnosis of exclusion by post-mortem examination. Intrathoracic petechiae, pulmonary edema, and occasionally signs of pre-morbid distress were found. All of these findings were non-specific and did not suggest any etiology for the sudden death of the infant. Preliminary analysis of quantitative determinations of the parameters studied in this proposal indicate a mechanism may be active in sudden infant death syndrome that is common to other known disease processes, namely chronic hypoventilation. Studies are planned to test the hypothesis that chronic hypoventilation, rather than an acute process, results in the death of infants from SIDS.

Proposed Course: Acquisition of sample (N=80), analysis of data; identification of previously unrecognized pathologic conditions which may be suggestive of mechanism of etiology and death.

NICHD Annual Report

July 1, 1973 through June 30, 1974
Perinatal Biology and Infant Mortality Branch

Contract and Collaborative Research

Contract Title: Development of Animal Model for Studies of Sudden Infant Death Syndrome (SIDS)

Contractor: University of Oxford
Nuffield Institute of Medical Research
Oxford, England

Money Allocated: No FY 74 funds (FY 73 funds: \$53,212,00 - 15 months.)

Objectives: The purpose of this contract is to develop an animal model for study of the sudden infant death syndrome (SIDS), using the lamb and the kitten, emphasizing autonomic mechanisms which interfere with spontaneous breathing. It is proposed to specifically define the nature of the materials which, when introduced into the larynx of newborn lambs, cause respiratory arrest. Substances to be evaluated will include, but will not be limited to, milk from different species, salt solutions of varying ionic composition (e.g., Na, K, Mg) and of varying osmolarity, stomach contents, and artificial feeding formulae. Responses of the lamb to the materials under investigation will be examined in relation to changes in consciousness and sleep, and with age from birth. In addition, pilot studies in kittens will be conducted to determine the feasibility of conducting experiments similar to those outlined for lambs. Graphic evidence to support the observations in lambs and kittens will be recorded throughout the critical tests.

Significance to Biomedical Research and the Program of the Institute: This project represents a logical and feasible extension of carefully documented studies presented earlier by these investigators which focus specifically on mechanisms which may cause sudden infant death syndrome. The animal species selected, (lamb and kitten), are particularly appropriate in that there is already a large and growing body of physiological information relating to them, much of it with reference to comparative data on the human fetus and newborn infant. Much of it has been developed by this group of investigators. If the cause of SIDS is multifactorial, this existing body of data and the combinations of systems and parameters in the research proposed should contribute significantly to the development of an animal model for SIDS and enhanced understanding about both the etiology and underlying mechanisms.

Proposed Course: Renewal for planned 3-year effort is anticipated.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : Sudden Death in Cats: A Spontaneously Occuring Model For Sudden Infant Death Syndrome (SIDS) in Man

Contractor : Washington State University, Pullman, Washington

Money Allocated : No FY74 Funds; (FY73 Funds \$145,300 - 18 months)

Objectives: The objective of this study is to develop an animal model for study of the sudden infant death syndrome, (SIDS), using kittens, with emphasis on developing lines of kittens which show high and low incidence of acute and unexplained death between two weeks and four months of age.

A genetic registry of kittens of various breeds for SIDS will be established. The karyotype of individual cats in (1) a randomly bred control group, (2) a high SIDS incidence group, and (3) a low SIDS incidence group of cats which were bred at Washington State University will be determined. A thorough pathologic workup will be conducted on those kittens that die suddenly and from unknown causes, between two weeks and four months of age, in the two groups of study kittens, and in appropriate littermate controls. This includes appropriate virologic and serologic studies for feline respiratory viruses such as Herpesvirus, Picornavirus, and Paramyxovirus in respiratory tissues and exudates. In addition, interferon production and its role in immune responses, antibody responses, immunoglobulin levels, and bacterial infections will be evaluated. From these results, the investigators will be able to determine if there is a genetic factor operative in causing sudden and unexplained kitten deaths. The epidemiologic data from these studies will be accumulated with a view to identify maternal factors associated with such deaths.

Significance to Biomedical Research and the Program of the Institute: This proposal is a multifacet approach involving genetics, infectious disease, immunology, and epidemiology to better understand naturally occurring, sudden unexplained deaths in kittens. The investigators have documented a SIDS-like syndrome in approximately 10 percent of 310 breedings of a group of cats who otherwise are normal. Groups of male and female cats have been identified that breed both SIDS prone and SIDS resistant kittens. The proposal to breed SIDS resistant and prone colonies of kittens along with random bred colonies for use in comparative studies is of significant scientific merit in relation to the Institute's sudden infant death program.

Proposed Course: Renewal for planned 3-year effort is anticipated.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Perinatal Biology and Infant Mortality Branch and
Epidemiology Branch
Contract and Collaborative Research

Contract Title: A Study of Monozygous and Dizygous Twins to Ascertain Possible Neurophysiologic Factors for the Sudden Infant Death Syndrome.

Contractor: Boston University
Money Allocated: \$33,764 (first year)

Objectives: Since there is good epidemiologic evidence that genetic factors are not important in SIDS this study will examine a number of neurophysiologic factors in newborn twin pairs at 3 days, 1 month, 2 months, and 3 months, and examine these factors for similarity across zygosity. Factors which significantly differ between dizygous twins but not monozygous pairs will be ruled out as possible risk factors for SIDS. A further objective of the study will be examination of the relationship between polygraphic recordings and those obtained from a behavior bed monitor.

Significance to Biomedical Research and Program of the Institute: Study results should provide indication of neurophysiologic factors which are more likely than other factors to repay further intensive study in order to elucidate etiology of SIDS. If behavior bed recordings reliably register polygraphic activity, then this device may prove a relatively cheap screening procedure.

Proposed Course: This contract has only been funded for the first year; subsequent funding will depend upon review.

A visit was made to Dr. Gould on January 28, 1974, by Mr. Howard Hoffman, and Drs. Charles R. Stark and Philip S. Spiers. At that time progress in the project was reviewed. Dr. Gould and his team had so far been able to perform polygraphic recordings on 9 twin sets - all of them of the dizygous form. It was reported that the respiratory measure of the Sander bed when used to classify sleep stage was correlated more than 95% with hand scoring techniques applied to traditional polygraphic records. It was the opinion of all members of the review team that the project was progressing satisfactorily.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Perinatal Biology and Infant Mortality Branch and
Epidemiology Branch
Contract and Collaborative Research

Contract Title: Development of Sleep and Cardiopulmonary Regulations within Sleep: Clinical Studies of a Functional Mechanism for Risk of Sudden Infant Death.

Contractor: University of Southern California

Money Allocated: \$141,372.00

Objectives: To describe the early maturation of sleep "states" and cardiopulmonary function during the various "states" in groups of newborns at high and low risk of the Sudden Infant Death Syndrome (SIDS).

Summary: In four groups of infants at high and low risk for SIDS, Drs. Hodgman and Sterman propose to collect large amounts of physiological data which will be used to evaluate the early maturation of sleep "states" and cardiopulmonary function during the various "states." The four study groups will be selected as follows:

1. Subsequent siblings of SIDS cases.
2. "Near miss for SIDS" cases.
3. In utero autonomic instability cases.
4. Healthy, "normal" newborns.

All study subjects except the "near miss" cases will be monitored during labor and approximately once each month during the first six months of life. The subsequent-sibs and low-risk subjects will also be monitored for one 12-hour period during the last trimester of pregnancy. Each monitoring session will last 12 hours and about 12 physiologic variables will be continuously recorded throughout the session.

This contract will be oriented exclusively toward data collection.

Progress: Three "near miss" cases have been enrolled and are under study as indicated in the workscope. Enrollment of sibs of SIDS and "control" pregnancies is on schedule with ample potential enrollees known to the contractor. All collected data has been forwarded to Dr. Sterman (see UCLA contract) for analysis. A detailed, on-site review of progress will occur May 6, 7, and 8, 1974.

Significance to Biomedical Research and Program of the Institute: At the present time the etiology of SIDS is quite unknown and considered new approaches must be attempted. This project is based on the knowledge that a very high proportion of SIDS events occur during periods of sleep. The working hypothesis is that extreme lability in the autonomic regulation of cardiopulmonary function during REM (active, rapid eye movement) sleep contributes a causal element common to nearly all SIDS events. To make

real progress in elucidating the etiology of SIDS, the Institute must open promising new avenues of research. It is hoped that this project will do so.

Proposed Course: Renewal for planned three year effort is anticipated.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Perinatal Biology and Infant Mortality Branch and
Epidemiology Branch
Contract and Collaborative Research

Contract Title: Analysis of State Integration and Related Cardiopulmonary Functions in Infants at High and Low Risk for the Sudden Infant Death Syndrome.

Contractor: University of California at Los Angeles

Money Allocated: \$234,300.00

Objectives: To describe the early maturation of sleep "states" and cardiopulmonary function during the various "states" in groups of newborns at high and low risk of the Sudden Infant Death Syndrome (SIDS).

Summary: In four groups of infants at high and low risk for SIDS, Drs. Hodgman and Sterman propose to collect large amounts of physiological data which will be used to evaluate the early maturation of sleep "states" and cardiopulmonary function during the various "states." The four study groups will be selected as follows:

1. Subsequent siblings of SIDS cases.
2. "Near miss for SIDS" cases
3. In utero autonomic instability cases.
4. Healthy, "normal" newborns.

All study subjects except the "near miss" cases will be monitored during labor and approximately once each month during the first six months of life. The subsequent-sibs and low-risk subjects will also be monitored for one 12-hour period during the last trimester of pregnancy. Each monitoring session will last 12 hours and about 12 physiologic variables will be continuously recorded throughout the session.

This contract will be oriented exclusively toward data analysis.

Progress: On May 6, 7, and 8, 1974, a detailed, on-site review of progress will be undertaken. The contractor is now in a "production" stage of data analysis, and it is anticipated that at the time of the May meeting all analyses specified in the workscope will be completed on virtually all data supplied by Dr. Hodgman (see University of Southern California contract) through March of 1974.

Significance to Biomedical Research and Program of the Institute: At the present time the etiology of SIDS is quite unknown and considered new approaches must be attempted. This project is based on the knowledge that a very high proportion of SIDS events occur during periods of sleep. The working hypothesis is that extreme lability in the autonomic regulation of cardiopulmonary function during REM (active, rapid eye movement) sleep contributes a causal element common to nearly all SIDS events. To make

real progress in elucidating the etiology of SIDS, the Institute must open promising new avenues of research. It is hoped that this project will do so.

Proposed Course: Renewal for planned three year effort is anticipated.

NICHD Annual Report
July 1, 1973 through June 30, 1974
Adult Development and Aging Branch

MISSION

The Congressional discussion that preceded the establishment of NICHD stated that the Institute would be responsible for the support of research on the biological, medical, and behavioral aspects of aging.

GROWTH OF EXTRAMURAL RESEARCH ON AGING

| <u>Fiscal Year</u> | <u>Research Grants</u> | <u>Contracts</u> | <u>Training Grants</u> | <u>Total</u> |
|------------------------|----------------------------|------------------|----------------------------|--------------|
| 1964 | \$2,744 | \$0 | \$302 | \$3,046 |
| 1966 | 3,219 | 59 | 1,451 | 4,729 |
| 1968 | 3,627 | 213 | 2,178 | 6,018 |
| 1970 | 3,226 | 485 | 2,305 | 6,016 |
| 1972 | 5,722 | 948 | 2,072 | 8,742 |
| 1974 (Est.) | 9,393 | 765 | 2,266 | 12,424 |

RESEARCH AND TRAINING PROGRAMS

In order to give an overview of the work underway in fiscal year 1974, research grants and program projects, contracts, postdoctoral fellowships, and research career development awards are classified together in substantive areas below. In this collation component projects of program projects (PP) are listed individually. The budgetary figures listed for the areas are estimates rather than final figures. Following the list of research activities, the training grants supported in fiscal year 1974 are listed. After the lists of grants and contracts there is a description of some aspects of the work being supported.

RESEARCH ACTIVITIES

GENERAL (\$223,036)

Maddox: Center for the Study of Aging and Human Development
Bell: Support of Collaboration among Longitudinal Aging Studies

ORGANISMIC MODELS OF AGING (\$408,650)

Rodents

Charles River Laboratories: Production of Aging Rats in an Isolator Environment
Charles River Laboratories: Maintenance of a Long-Term Aged Rat Colony
Charles River Laboratories: Development of a Colony of Mice for Research in Aging
Cornell University: Summer Institute on Pathobiology of Aging in Experimental Animals

Invertebrates

Rothstein (PP-Rothstein): Studies of Aging in a Nematode Model System
Hirsh (PP-Ham): Studies on Aging with the Small Nematode, Caenorhabditis elegans
Siakotos: Developmental Changes in Dipteran Lipopigments

AGING IN RAPIDLY DIVIDING DIPLOID CELL SYSTEMS (\$1,500,157)

Absher: Studies of Aging of Human Diploid Fibroblasts in vitro
Bethel (PP-Seifter): The Biosynthesis of Collagen and Other Proteins in Aging Fibroblasts
Blumenfeld (PP-Seifter): The Composition, Immunological Character, Enzymatic and Transport Activities of Cell Membranes of Aging Fibroblasts
Brandes (PP-Cristofalo): Aging of Human Diploid Cells in vitro
Comings: Aging and DNA—in vitro Studies
Cristofalo (PP-Cristofalo): Protein Function and Its Modification by Hormones and Growth Factors during Aging in vitro
Dallman (PP-Packer): Nucleic Acid Metabolism of Human Fibroblasts Grown in vitro
Daniel: Studies on Aging of Mouse Mammary Epithelium
Deamer (PP-Packer): Peroxidation and Human Fibroblasts Grown in vitro
Diamond (PP-Cristofalo): Transformation Studies
Ham (PP-Ham): Environmental Influences on Cell Aging and Clonal Culture Experiments
Harrison (PP-Russell): Age Changes in Functional Capacity of Hemopoietic Stem Cells
Hayflick: The Production, Distribution, and Study of Human Diploid Cell Strains
Hayflick: Programmed Senescence in Cultured Human Cells
Hoyer (PP-Cristofalo): Transcriptional Changes in Transformed and Early-Late Passage Diploid Cells
Klevecz: Temporal Order and Aging in Cultured Somatic Cells

Makman (PP-Seifter): Adenyl Cyclase System and Hormone Receptors in Aging Fibroblasts
 Miller: Aging and Carbohydrates of Fibroblasts
 Moorehead (PP-Cristofalo): Genetic Factors and Aging of Cultured Somatic Cells
 Packer (PP-Packer): Mitochondrial Biogenesis in Human Fibroblasts Grown in vitro
 Packer (PP-Packer): Fine Structure of Human Fibroblasts Grown in vitro
 Packer (PP-Packer): Fractionation of Human Fibroblasts Grown in vitro
 Porter (PP-Ham): Ultrastructural Studies and Electron Microscopy
 Prescott (PP-Ham): Hybridization Studies with Enucleated Cytoplasm and Isolated Nuclei
 Sabatini (PP-Cristofalo): Studies on Translation and Macromolecular Turnover in Cellular Aging
 Tappel (PP-Packer): Lysosomal Activity of Human Fibroblasts Grown in vitro

ENDOCRINE CHANGE WITH AGING (\$340,122)

Ovarian Aging and the Menopause

Leathem: Ovarian Involution in Aging
 MacDonald: Estrogen Studies in the Postmenopausal Woman
 Piacsek: Effects of Aging on the CNS: Pituitary: Gonadal Axis
 Saville: Prevention of Bone Loss in the Menopause
 van den Noort: Estrogen Therapy and Stroke Risk in the Postmenopausal Woman

Other Endocrine

Hayashida: Studies with Growth Hormone and Prolactin
 Litwack (PP-Adelman): Properties of Hormone-Binding Macromolecules in Liver, Brain, and Other Tissues during Development and Aging
 Romanoff: Effects of Aging on Steroid Metabolism in Man

CONNECTIVE TISSUE AGING (\$942,611)

Baer (PP-Robbins): Structure-Property-Age Studies of Collagen Fiber Bundles
 Baylink: Bone Quality in Patients with Osteoporosis of Aging
 Bensusan (PP-Robbins): Connective Tissue Aging
 Bornstein: The Influence of Age on the Nature and Location of Interchain Covalent Cross-Links in Human Collagen
 Davison (PP-Sanadi): Cross-Links in Collagen and Aging
 Federman: Changes in Cell Type and Structure in Developing Bone
 Frankel (PP-Robbins): Aging of Neuro-Musculo-Skeletal System
 Franzblau (PP-Sinex): Studies on Connective Tissue Proteins and Their Relationship to Aging
 Fry: Aging Phenomena in Periarticular Tissue and Articular Cartilage
 Gallop (PP-Gallop): Quantity and Chemical Nature of Collagen Synthesized by Fibroblasts and Other Connective Tissue
 Gallop (PP-Gallop): Alterations in the Molecular Biology of Collagen Accompanying Development, Maturation, Aging, and Disease
 Geil (PP-Robbins): Collagen Fibril Ultrastructure and Deformation
 Hamlin (PP-Robbins): Chemistry of Aging Human Collagen

Klein: Collagen Biochemistry during Bone Remodeling and Aging
Klein (PP-Robbins): Biological Turnover and Cross-Linking of Aging Collagen
Meyer: Mucopolysaccharides of Connective Tissues
Ortner: Various Aspects of Aging in Human Bone
Tonna: Skeletal Cell and Matrical Changes during Aging
Walton (PP-Robbins): Elastin Structure and Aging

IMMUNOLOGICAL CHANGES IN AGING (\$559,494)

Baum: Immunological Studies in an Aging Population
Buckley (PP-Maddox): Immunologic Studies of Older Persons
Claman: Immunological Changes in Aging
Franklin: Immunologic Studies of Amyloid Accompanying Aging
Gelfant: Role of Immunocytes and Immunosuppressants in Aging
Harris: Aging and Immune Responses to Transplants and Tumors
Phair: Host Defenses and Auto-Immunity in the Aged
Segre: Parameters of the Immune Response in Aged Mice
Smith: A Study of Accelerated Immunological Aging
Teague: Age-Associated Involution of Cellular Immunity in Mice
Tyan: Immune System Stem Cells—In Ontogenesis and Aging
Walford: Immunology and Aging
Yunis: Immunogenetics of Aging in Man

OTHER BIOLOGICAL ASPECTS OF AGING (\$1,427,103)

General

Gerontological Society: Symposia on Biology of Aging at Gerontology Society Meetings
Gordon Foundation: Partial Support of Gordon Conference on Aging

Mitochondria

Koukol (PP-Sanadi): Control of Energy Metabolism in Aging
Sanadi (PP-Sanadi): Mitochondrial Reactions to Senescence
Tymoczko: The Effect of Aging on Mitochondrial DNA

Nucleic Acids and Chromosomes

Bell: Gene Transfer during Differentiation and Aging
Benson: Control of Transcription in Aging Animals
Chang: Role of Transfer RNA in Cellular Aging
Griebel (PP-Sinex): Organization of Chromatin—Protein and RNA during Aging
Huang: Nucleo-Cytoplasmic Interactions during Development and Aging
Lang: Metals and Nucleic Acids in Growth and Aging
Mays: Relation of tRNA to Protein Errors in Aging Rat Liver
O'Meara: Age-Related Changes in Chromatic Proteins
Paik (PP-Adelman): Histone Metabolism during Aging
Taylor (PP-Russell): The Effect of Maternal Age on the Incidence of Chromosomal Anomalies

Membranes

- Barber: Membrane Changes during Aging and Vitamin E Deficiency
Masoro (PP-Masoro): Studies on the Plasma Membrane of the Adipocyte
Masoro (PP-Masoro): Changes in the Sarcoplasmic Reticulum Membrane with Age
Roberts (PP-Masoro): Changes in Cardiac Membrane Properties with Age
Widnell: Membrane Function in Aging and Development
Zimmerman (PP-Masoro): The Influence of the Aging Process on the Function of the Neural Cell Membrane

Other

- Adelman (PP-Adelman): Age-Dependent Control of Enzyme Adaptation
Adelman: Effects of Senescence on Enzyme Regulation
Bayer (PP-Eichorn): Physical Health and Physiologic Functioning
Bodenstein: Aging, Its Relation to Cell Growth and Differentiation
Busse (PP-Maddox): The Effect of Aging upon the Nervous System: Longitudinal Studies of the Physical, Psychological, and Social Effects of Aging
Ensinck: Influence of Aging on the Biosynthesis and Secretion of Insulin in Mammals
Finch: Aging and Biosynthesis in Non-Dividing Cells
Finch: Gene Function during Post-Natal Development and Aging
Gershon: Faulty Protein Molecules in Senescence
Goldthwaite: Regulation of Leaf Senescence by Plant Hormones
Grande: Effect of Age on Adipose Tissue Lipolysis
Hanson (PP-Adelman): Aspects of Metabolis Regulation in Developing Mammalian Liver
Hollozy: Exercise-Induced Biochemical and Anatomic Adaptations
Hruza: Metabolism of Lipids and Calcium during Aging
Kaldor (PP-Masoro): Influences of the Aging Process on Muscle Contractile and Modulator Proteins
Lansing (PP-Lansing): Experimental Cytology of Aging Cells and Tissues
LoSpalluto: Lymphoid Cell Function and Aging
Lowenstein: Aging in the Kidney
Martin: Consequence of in utero Treatment on Aging of Progeny
Meadow: A Study of Sugar Transport in Bacteria
Myers (PP-Russell): Genetic Differences in Aging of Mice
Polgar: Participation of Cyclic AMP System in Cellular Aging
Pond: Effect of Dietary Pufa-Vitamin E on Dam and Progeny
Poole (PP-deDuve): Cellular Rejuvenation and Aging
Sacher: Tissue Aging and Senescence: Control of Enzyme Activity
Scrimshaw: Protein Needs of Elderly People
Sreter: Biochemical Changes in Development and Aging Muscles
Stoffolano: Effect of Aging on Chemoreceptor Sensillia
Willis: Developmental Analysis
Wright: Computer Analysis of Differentiation

NEUROLOGIC CHANGE WITH AGING (\$352,224)

- Bondareff: Age Changes in the Neuronal Microenvironment
Eleftherion (PP-Russell): Age Changes in the Brain of Inbred and Hybrid Mice

Epstein: The Molecular Basis of Neuromuscular Development
Harrison (PP-Sinex): Assessment of Auditory Behavior as a Function of Age
Hinds (PP-Sinex): Aging in the Olfactory Bulb
Marsh (PP-Maddox): Evoked Potentials in Brain Function and Aging
McNary (PP-Sinex): Aging of the Microcirculation of the Auditory System
Peters (PP-Sinex): Age-Related Changes in the Auditory Cortex
Pieringer (PP-Adelman): The Biochemistry of the Maturation of New Tissue
Schiffman: Gustatory and Olfactory Quality Changes with Age

DISEASE ORIENTED RESEARCH (\$380,436)

Appel (PP-Maddox): Organic Approaches to Dementia
Bowers (PP-deDuve): Subcellular Events in Cellular Immunity
Glomset (PP-Bierman): Plasma Lipoprotein Metabolism
Kohn: Aging of Connective Tissue: Age and Neoplasia
Muller (PP-deDuve): Subcellular Events in Arterial Disease
Paffenbarger: Factors in Youth Predisposing to Chronic Diseases
Parker (PP-Bierman): Electron Microscopic Radioautographic Studies on Free
Cholesterol in Primate Atherosclerotic Plaques and
Xanthomas

COGNITIVE CHANGE WITH AGE AND SENILE DEMENTIA (\$916,659)

Botwinick: Psychophysiological Aspects of Aging
Davis: Aging in Monkeys
Eisdorfer: Learning and Psychophysiological Studies in the Aged
Elias: Age Effects on Verbal and Non-Verbal Processes
Fonda: Gaba Metabolism—A Biochemical Key to Mental Aging
Fozard: Mental Performance and Aging
Erwin (PP-Maddox): Studies of the Psychophysiology of Aging
Kinsbourne (PP-Maddox): Age Effect on Multiple-Cognitive Processes
Labouvie: Modifying Intellectual Behavior in the Aged
McGaugh: Psychophysiology of Memory and Aging
Mistler-Lachman: Changes in Semantic Memory in Maturity and Old Age
Schaie: Cognitive Behavior in Maturity and Old Age
Smith: Interaction between Human Aging and Memory
Thompson (PP-Maddox): Hyperbaric Oxygenation, Behavior and EEG in the Aged
Thompson (PP-Maddox): Learning and Psychophysiological Parameters in the Aged
Vanderplas: Incremental Cognitive Functions in Aging

OTHER PSYCHOLOGICAL RESEARCH (\$398,057)

Crockett: Personal Constructs, Aging, and Impression Formation
Eichorn (PP-Eichorn): Levels and Patterns of Mental and Physical Development
among Kinships
Haan (PP-Eichorn): Ego Functioning, Coping and Defense
Haan (PP-Eichorn): Sameness and Change in Personality Development
Haan (PP-Eichorn): Relationships among Ego, Moral, and Cognitive Operations
and Political Social Views
Langer (PP-Eichorn): A Longitudinal Study of Operational and Moral Reasoning
in Families
Lowenthal (PP-Lowenthal): Longitudinal Study of Adaptive Behavior

Nowlin (PP-Maddox): Arousal Patterns in a Structured Interpersonal Setting—
Age-Related Differences
Peskin (PP-Eichorn): Stage Developmental Antecedents of Adult Psychological
Health
Riegle: The Effect of Aging on Hypothalamic Control Systems
Skolnick (PP-Eichorn): Psychological Correlates of Linguistic Style
Skolnick (PP-Eichorn): Variation among Marriages
Skolnick (PP-Eichorn): Participation in Social Networks
Sprott (PP-Russell): Age-Dependent Behavioral Changes in Inbred and Hybrid Mice
Whissel-Buecky (PP-Eichorn): Investigation of the Genetic Basis of Continuous
Variation

SOCIETAL ASPECTS OF AGING (\$458,230)

Clark (PP-Lowenthal): Ethnic Identity and Adult Development
Eichorn: Intergenerational Studies of Development and Aging
Eisdorfer: Summer Institute on Psychological and Social Aspects of Aging
Kalish: Social Gerontology of Religion and the Clergy
Myers (PP-Maddox): Studies of the Social Aspects of Aging in Human Development
Myers: Studies of Models for Forecasting Future U.S. Population
Ostfeld: Relocation in Old Age—Health and Psychosocial Impact
Silverman: A Cross-Cultural Study on the Treatment of the Aged

TRAINING GRANTS (\$2,178,627)

Psychology and Social Sciences

Botwinick: Aging and Development
Eisdorfer: Research in Aging
Loeb: Social Gerontology
Meyer: Developmental Psychology: Development and Aging
Neugarten: Gerontology
Peterson: Inter-University Training in Social Gerontology
Reese: Lifespan Developmental Psychology
Rosenberg: Training in Social Gerontology
Taylor: Interdisciplinary Training in Gerontology

Biological Sciences

Horvath: Physiology of Exercise and Stress
Huang: Age-Dependent Nucleo-Cytoplasmic Interaction
Kohn: Aging of Mammalian Tissues
Rockstein: Gerontology
Sanadi: Biochemical and Biophysical Basis of Aging
Sinex: Biochemistry of Aging
Timiras: Developmental Physiology and Aging

Mixed Biological, Psychological, and Social Sciences

Birren: Comprehensive Training Program in Gerontology
Maddox: Behavior and Physiology in Aging and Human Development

GENERAL

The Branch supports by reimbursable agreement with the Veterans Administration a series of yearly meetings to coordinate certain longitudinal studies on aging. This series of meetings was the mechanism finally worked out to coordinate such studies following a review of overlap and gaps in such studies requested by the House Committee on Government Operations. The management of these meetings is carried out by Dr. Benjamin Bell who is associated with the VA Normative Study of Aging. Representatives from this study and from a number of other longitudinal studies of aging attend these meetings, attempt to standardize their measurements so that data collected will be comparable, consider what areas in which pooling of data might be useful, and discuss what other problems they have in common.

NICHD has authority to make grants designated as Center Grants to institutions to facilitate, develop, and strengthen their capability for conducting an organized program of research on aging processes. Center Grants are concerned with support for scientific personnel and administrative management, common equipment, central support services, and development of new research areas and opportunities. NICHD policy has been to make such Center Grants only to institutions where a strong program of NICHD-supported research has been developed. Only one such award has been made and that to Duke University School of Medicine.

ORGANISMIC MODELS OF AGING

Mammalian Models for Aging Research

Understanding of most of the aspects of the biology of man has been based to a very large extent on studies of the biology of other living creatures. It appears likely that an understanding of the biology of aging in man will provide no exception to this general rule. A number of advantages can be expected to derive from the study of aging in other species. The study of aging can be compressed into a shorter period of time by studying species with a short lifespan. Experimental procedures can be undertaken that would not be permissible in man. Particular species may, through some particular circumstance, highlight some particular aspects of aging.

During recent years the extramural aging program has explored through conferences, workshops, and personal exchange with investigators relevant factors in the selection, development and provision of suitable animals for experimental work on aging. Most consideration has been given to the use of rats and mice since these are well studied animals, widely used by investigators in many fields. They are particularly useful in aging research since they have short lifespans and are small and thus relatively inexpensive to raise to senescence. However, other species such as insectivores, dogs, and subhuman primates have been considered.

In recent years methods have evolved that along with older methods permit the development of rodents very well suited to aging research. By technology

involving cesarean section and the use of barriers or isolators that exclude unwanted bacteria it is possible to raise rats and mice free of pathogens and with a defined bacterial flora. In addition both inbred and outbred lines of rats and mice are available. The genetics of the mouse has been particularly well studied.

For the past four years the contract mechanism has been used to establish colonies of aging rats and mice at the Charles River Laboratories. The animals are well defined genetically and with respect to their bacterial flora. Sample animals are autopsied at intervals so that they are also well defined pathologically. Their life-tables are being established on the basis of spontaneous mortality in the colonies. These animals can be purchased by investigators involved in aging research. The funds, of course, usually come from research grants.

Many of the aspects of the use of rats and mice in aging research were described in the publication "Development of the Rodent as a Model System of Aging" published by NICHD and based on a series of conferences held by the extramural aging program.

Invertebrate Models for Aging Research

One of the difficulties encountered in experimental studies of aging in mammals is that even the short-lived mammalian species usually live three years or longer. This tends to set a slow pace for the sequential development of data and concepts. Thus a number of investigators in this century have studied aging in short-lived invertebrates. Since many of the cellular and subcellular processes occurring in metazoa of all degrees of complexity are similar, it appears possible that some metazoan aging processes at those levels may be similar.

For this reason, over the years, the Branch has supported a number of conferences to discuss invertebrate models of aging. Currently it is supporting a small number of studies on aging in nematodes and aging in insects.

AGING IN RAPIDLY DIVIDING DIPLOID CELLS

In 1953 Swim and Parker published the first report stating that cells cultured from a variety of human tissues had only a finite capacity for reproduction by division. In 1961 their findings were confirmed by Hayflick and Moorehead in studies of fibroblasts obtained from human embryonic lung. They found that these cells would pass through about 50 population doublings before dying. The view that emerged from their consideration of the problem was that human cells that retained a normal karyotype had a finite generational capacity. It had become obvious from earlier studies by many investigators that some malignant cells with abnormal karyotypes and some cells that acquired karyotypical abnormalities in tissue culture appeared to have no limit to their generational capacity.

The cells that have been used in studying this limitation of generational capacity in tissue culture undergo many more divisions than they would ever naturally undergo in the intact animal. However, Daniel has produced a similar phenomenon in intact animals by transferring mammary epithelium from young mice through series of young mice whose own mammary epithelium has been removed.

The production of a large number of new cells is induced on each transfer. These cells, after undergoing more reproductive divisions than they ever would under natural conditions, lose the ability to divide reproductively and thus to create new mammary glands.

Thus it appears that the limitation on generational potential that occurs in tissue culture can be elicited experimentally in the whole animal. However, the relation of this to aging processes that occur in the intact animal still remains obscure. The observation that the generational capacity of human fibroblasts varies inversely with the age of the donor suggests that the process that limits generational potential may occur in vivo but at a much slower rate than in vitro.

Studies are now underway to determine what biochemical and morphological changes occur as fibroblasts lose their ability to divide. It appears possible by suitable techniques to fuse young nuclei with enucleated old cells and vice versa. Studies using this technique are now in progress and may indicate whether the mechanisms involved are in the nucleus or in the cytoplasm.

ENDOCRINE CHANGE WITH AGE

Changes with function with increasing age occur in a number of the endocrine glands and the Branch supports a small number of grants and contracts in this area. The major programmatic efforts that have been made in this area have dealt with the menopause and postmenopausal state.

The female reproductive system undergoes major changes during the course of its development and decay. After a period of relative inactivity during childhood, ovarian activity increases dramatically at puberty. The body produces eggs and ovarian hormones in a strictly patterned reproductive cycle which, unless interrupted by pregnancy, disease, or hormonal contraceptives, repeats itself with little variation until about the age of 50. At that time, and over a period of several years ovarian activity falters and then almost completely ceases.

The hormones secreted by the ovaries at menarche and through the reproductive years exert a profound influence on the maturation and maintenance of the breasts and sexual organs. The great reduction in the ovarian excretion of estrogen during the menopause and in the postmenopausal years causes atrophic changes in tissues previously stimulated by estrogen. In addition, hot flashes and drenching sweats may occur. The severity of these symptoms varies greatly among women and is probably related to the degree of estrogen deprivation. This, in turn, depends on how much function the ovary retains and how much estrogen is produced by tissue and organs other than the ovaries. It has been thought that other types of deterioration, such as osteoporosis and atherosclerosis, become worse with estrogen deprivation. The evidence for this, however, is not strong.

It has been known for several decades that the flashes, sweats, and genital atrophy associated with the menopause can be eliminated by the administration of estrogenic hormones. However, a great deal remains to be learned about who needs this type of treatment, what the best methods of treatment are, and what the undesirable results of such therapy may be. Complications, particularly thrombosis and stroke, have been caused by similar hormones used as contraceptive

agents. There is some evidence that these complications are more frequent in older women of reproductive age. It is possible that they may be even more common in postmenopausal women.

The activation of the ovary at puberty is initiated by pituitary hormones produced under the control of the central nervous system. The cessation of ovarian activity at the menopause appears to be due to ovarian failure. The mechanism of this is unknown, but it does provide an example of aging in a discrete organ proceeding at a rate much greater than the average rate of the rest of the body.

The Branch is enlarging its program dealing with ovarian aging and the management of the postmenopausal state. Its major effort at present is the support by contract of a retrospective study of the effect of estrogen therapy on the incidence of stroke in postmenopausal women.

IMMUNOLOGIC CHANGES IN AGING

The immunological changes that occur with age represent an important area for study because they are large and cause serious problems in the functioning of the elderly, and because when sufficient knowledge about immunologic function and its changes with age have accumulated they may prove to be amenable to therapy. The progressive changes that take place in immunologic function are especially interesting in that the immune system reaches peak development and begins its progressive decline with age before many other systems have reached maturity. Immune competence at birth is low, reaches a maximum value at about ten years of age, and thereafter declines until in old age it is about only ten percent of its previous highest value.

The importance of the immune system as a defense against infectious diseases has long been known. The elderly with their declining immune responses are particularly susceptible to infectious diseases. Much evidence has accumulated indicating that the ability to tolerate a number of infections is greater in childhood than in the adult years. In addition to its role in preventing infectious diseases, it is also recognized that immunologic competence may play an important role in preventing the development of cancer and of autoimmune diseases. All this assumes considerable importance for the middle-aged and elderly since they become increasingly susceptible to all these pathological processes--infectious diseases, cancer, and autoimmune diseases--as they get older. The development of means of augmenting immune competence in the later years of life would represent a major advance in improving the health of older people.

The Branch supports a small program of research on immunologic change with age and is expanding this program.

CONNECTIVE TISSUE

At the gross level there are striking alterations with age in bodily parts composed largely of connective tissue. Skin thins and becomes inelastic, arteries dilate and lose elasticity, cartilage becomes friable, bones rarefy, and tendons lose tensile strength. These connective tissue changes are responsible for much

of the outward appearance of the elderly, for rupture of tendons, for arthritic changes, and for arterial deterioration.

These gross changes appear to arise in large part from alterations in the collagen, elastin, and mucopolysaccharides that form the connective tissue. This is true even in bone whose minerals are contained in a matrix made of collagen and mucopolysaccharides.

The collagenous and elastic structures consist of complex molecules laid down outside the cells and in large part replaced slowly or not at all. It is not surprising that deleterious changes should occur in such non-renewed aggregates of complex molecules with time. Some of the collagen and much of the mucopolysaccharide matrix, on the other hand, is more rapidly renewed. It appears probable that changes in the content of these fractions is related to changing cellular activity.

The Branch supports a moderate amount of research dealing with changes in collagen and bone with age but almost no research on elastin or mucopolysaccharides.

OTHER BIOLOGICAL ASPECTS OF AGING

The Branch supports a moderately large amount of research on biological aspects of aging other than those singled out for special discussion in this report. This other research is heterogeneous in one sense but remarkably homogeneous in another in that it is almost all directed at changes that occur at the cellular, subcellular, and molecular levels. Research is being supported on cellular and subcellular membranes, mitochondria, lysosomes, cytoplasmic and nuclear nucleic acids, and protein metabolism. It seems probable that it is at this level that scientists will find age changes that are common to many multicellular animals and that are responsible for many manifestations of aging at the organismic level.

COGNITIVE CHANGE WITH AGE AND SENILE DEMENTIA

Under this heading is included studies of the cognitive changes that occur with age and the morphological, physiological, and biochemical changes in the central nervous system which probably underlie them.

There are major changes in mental function across the years. Knowledge about these is based not so much on sophisticated scientific study as on every-day observation. The speed with which children learn (and forget) new languages in contrast to adults, the importance of early entry into many types of activity in determining later competence, the greater creativity of the years of late adolescence and early adulthood, and the senile dementias attest to changes in mental function with age. However, this common knowledge is not the result of controlled experimentation, and may in many ways be misleading. Uncontrolled and even unrecognized variables may be operating that make most of what we think we know about this field unreliable.

Most studies of intellectual competence as a function of age have been cross-sectional--that is, they have compared the function of individuals of different

ages at the same point in time. These studies have shown a decline in intellectual capabilities with age. The declines in general have been small with tests of verbal ability and larger with tests of problem-solving ability. Recently, longitudinal studies have cast doubt on these findings. When the same individuals are tested at two points in time making possible measurement of the decline in each of many different individuals, there may be little or no decline in the scores obtained on various tests said to measure some aspects of intelligence. An exception to this occurs in some patients with demonstrable abnormalities. For example, in later life what is measured as intelligence has been shown to decline in subjects who have cardiovascular hypertension.

It is apparent that this area of knowledge is in an unsatisfactory state. Studies showing no loss in intelligence have to be reconciled with what would be expected on a basis of the generalized brain atrophy that occurs with increasing age and with the fact that an appreciable fraction of older people have such severe loss of mental function that they are considered demented.

Senile dementia was once considered to be due mainly to cerebral arteriosclerosis. Most neuropathologists now think that this is not the case and that loss of function with age is a result of neuronal changes not dependent on vascular inadequacy. Most of the patients with senile dementia and many with dementia occurring in the middle years of life have pathological changes usually considered characteristic of Alzheimer's disease. However, it is not clear whether this is a disease or simply aging of the brain occurring usually late in life but occasionally somewhat prematurely.

The Branch supports a moderate amount of research on the cognitive changes that occur across the span of the adult years and some research on neurophysiological changes occurring as the central nervous system ages. It supports almost no research aimed at understanding the processes that occur in the severe dementing processes of the elderly. This represents a serious gap in the NICHD program on aging.

OTHER PSYCHOLOGICAL RESEARCH

Psychological research other than that dealing with cognitive processes supported by the Branch includes studies of how the elderly are integrated into society or separated from it in different ethnic groups, cross-cultural studies of the effect of different societies on personality functioning in later life, and psychosocial studies of the transitional phases between the different stages of life.

SOCIETAL ASPECTS OF AGING

The average duration of life has changed greatly since the evolutionary emergence of mankind, and there have been corresponding changes in the many different populations that have existed on the earth. There is no reason to think that population age-structures will not continue to change, and it is possible that they will be exaggerated by major changes in the average length of life. The human race has adjusted to a great variety of age-structures successfully in the past and must continue to do so in the future.

Although mankind has adjusted successfully to many different population sizes and age-structures, these demographic characteristics may provide major challenges to societal function in the future. The severity of these challenges will be determined primarily by future fertility and mortality rates.

Since fertility rates may be lower in the future than they formerly were, whatever changes in mortality rates that occur may assume more importance than they would have in the past. For example, with zero population growth, current mortality rates, and no migration, this country would ultimately have 16 percent of its population over the age of 65 years. Zero population growth combined with the improvement of mortality rates that may come with control of vascular disease and cancer could lead to an even larger fraction of the population exceeding 65 years of age. For these reasons the Branch supports research to investigate the implications of changes in mortality rates for society and for the individuals who compose it.

It is, of course, impossible to predict future mortality rates. However, various assumptions can be made and their implications evaluated in terms of the options available for society in the future. The research currently supported by the Branch is concerned with the evaluation of existing mathematical models and the implications that can be drawn from them at this time. Although some idea of the range of possibilities has been obtained by investigating rather extreme assumptions, much more must be done before a comprehensive view of the possibilities facing society can be formulated.

NICHD Annual Report

July 1, 1973 through June 30, 1974

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: HD-3-2725

Contract Title: Contract to Breed, Rear and Maintain a Colony of Inbred Aging Laboratory Rats for Aging Research (Modified)

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: \$86,890.00 (FY 1973)

- Objectives:
1. Meet current and projected demands for senescent laboratory rats reared on a defined diet in a specific pathogen-free environment.
 2. Establish a standing commercial resource of senescent rats on which investigators can immediately draw for aged laboratory rats.
 3. Develop baseline physiological and pathological characterization of the Fischer 344 rat over their full lifespan.
 4. Establish survival curves for laboratory rats reared specific pathogen-free behind a defined barrier system.
 5. Increase the numbers and ages of animals to be made available for studies in aging.

Significance for Aging Research: A major constraint influencing the development of aging research has been the almost total absence of an aged animal resource sufficiently characterized to meet the unique needs of aging research. The development of a colony of aging laboratory rats under this contract will significantly enhance the quality and quantity of aging research by providing aged animals that are reared in a defined environment on a standardized diet, free of pathogenic organisms, and characterized with regard to age-specific causes of death.

Basic to the development of studies in aging research in animals is a characterization of expected physiological and pathological changes that may occur over the animals' full lifespan as well as life tables that accurately reflect survival at specific ages. A primary aim of this contract is to acquire this data and make it available to investigators in aging. With this information, a reasonable comparative assessment can be made as to whether the animals, strain or stock is suitable for studies in aging. Also, within reasonable limits, numbers of animals needed for statistical significance of studies can be readily established, thus minimizing the likelihood of supporting excessive numbers of animals or too few animals for statistical significance of the study.

Currently many investigators in aging cannot acquire aged animals short of rearing the animals themselves, nor are they able to maintain aging rats under the laboratory conditions necessary to allow the animals to survive long enough to observe truly senescent change with age. Also, competent young investigators more often than not cannot support aging colonies of rats until they successfully compete for research support. Without this resource many imaginative young investigators will continue to be excluded from research in aging simply because they are unable to identify an aged animal resource which they could use in the studies they propose in aging. The country would ultimately have 10 percent of its population over the age of 65 years. Zero population growth is a goal that may come, but it is not a goal that should be pursued. Proposed Course: Contract is to be continued for a minimum of two years with the contract becoming increasingly self-sustaining, and until age-specific causes of death and life tables for the colony and strain can be established. For these reasons the Branch is searching to investigate the implications of changes in mortality rates in society and for the individuals who compose it.

It is, of course, impossible to predict future mortality rates. However, various assumptions can be made and their implications evaluated in terms of the options available for society in the future. The research currently supported by the Branch is concerned with the evaluation of existing mortality models and the implications that can be drawn from them at this time. Although some idea of the range of possibilities has been obtained by investigating rather extreme assumptions, much more must be done before a comprehensive picture of the possibilities facing society can be formulated.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: HD-4-2811

Contract Title: Development of a Production Colony of Three Genotypes and a Non-Inbred Strain of Laboratory Mouse for Aging Research

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: \$85,000.00 (FY 1974)

- Objectives:
1. Provide characterized genetically defined strains of laboratory mice reared in a defined environment for research in aging.
 2. Develop a ready commercial source of aging mice of three basic genotypes to meet the demands for aging laboratory mice.
 3. Minimize lag time for the development of studies in aging requiring aged genetically defined laboratory mice from a controlled environment.
 4. Provide the minimum number of strains of mice necessary for cross comparison and extrapolation of experimental results to a broader natural population.
 5. Develop a colony of laboratory mouse strains in which pathological processes, degenerative change, morbidity and mortality to age 24 months are largely known and predictable.

Significance for Aging Research: A lack of aged genetically and biologically defined animals reared in a controlled environment has long hampered the development of aging research, particularly in the field of immunology. With increasing frequency studies in aging research require animals of known genetic background, biological characterization and environmental status. To meet this need for strains of genetic specificity, diversity and generalizability a colony of aging mice of the inbred strains C57/BL6, BALB/c, the inbred F₁ hybrid of the two inbred strains was established in a barrier enclosure (SPF) at Charles River Breeding Laboratories. Profile data will be acquired on the colony and strains of animals by periodic sacrifice and necropsy.

The major significance of this contract is the development of a readily available resource of aging, genetically defined and characterized strains of laboratory mice reared in a controlled environment. The standing colony of aging mice of the three genotypes proposed under this research contract provides investigators in aging with basic genetically controlled model systems previously unavailable to most investigators in aging. This has moderated one of the primary constraining influences on the development of aging

research in animals by making available: 1) basic genetic model systems of the aging laboratory mouse for studies in aging requiring specific genetic control, 2) for study, one or several comparative animal model systems within a species, 3) an animal of known biological characterization and environmental status.

Proposed Course: Contract will continue for a minimum of one year with the contract becoming increasingly self-sustaining in subsequent years.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: HD-4-2843

Contract Title: Production Colony of Aging Rats in an Isolator Environment

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: \$47,924.00 (FY 1974)

- Objectives:
1. Establish a production, rearing and maintenance system for aging rats that prevents introduction of pathogens prematurely shortening the natural lifespan of aging laboratory rats.
 2. Determine the commercial and research feasibility as well as per animal cost effectiveness of isolator rearing of aging laboratory rats compared with barrier and conventionally reared rats.
 3. To develop for research in aging:
 - a) a limited source of aging rats free of bacterial and detectable viral diseases.
 - b) a system of environmental control that is standardized and transferable to the research laboratory without unduly compromising the biological integrity of the animals.
 - c) laboratory rats that are known to survive to natural senescence as a natural population independent of bacterial diseases or detectable viral diseases.
 4. Compare survival, pathology and degenerative change in isolator reared rats with barrier and conventionally reared animals.

Significance for Aging Research: The lack of aging experimental animals of defined quality is one of the major limiting factors to the study of aging, particularly animals that survive to natural senescence. Until recently aging studies in animals, especially rodents, were limited to those animals hardy enough to survive the stress of disease and a fluctuating physical environment. During the past several years several methods have evolved in the development, husbandry, maintenance and care of laboratory animals that permit routine cesarean derivation and rearing of laboratory rats and other experimental animals behind a barrier or in the rigidly controlled environment of plastic isolators. The isolator excludes the introduction of bacterial and detectable viral agents. This methodology combined with genetically defined animals on a stabilized diet and a close monitoring system goes far toward establishing and providing aging animals that survive to natural

senescence independent of the complications of infectious disease, parasitism, or wide variability in physical environment.

Basically the contract for an isolator reared colony of aging rats is concerned with developing an aging rat colony in a rigidly controlled isolator environment free of bacterial agents, except those purposefully introduced as normal bacterial flora. Changes in the aging process can most readily be determined when environmental conditions are uniformly controlled and sufficient numbers of animals representative of the total population of animals survive to an aged condition that can be studied as characteristic of the normal processes of aging. For this reason the contract requires uniformity in environment, diet, humidity and temperature and genetic quality of the animals in the isolator.

Once it is clearly established that laboratory rats can be maintained for their full lifespan independent of detectable microbial disease, and survival rates largely offset added costs for isolator maintenance, a resource of isolator reared animals can be provided to investigators in aging. The need for developing expensive and sophisticated environmentally controlled buildings to support the colony is unnecessary since the total environment of the animal is transferred within the isolator unit.

Studies of aging independent of environmental variables and disease can then be conducted to increase understanding of aging processes in animals and how these processes may apply to man or lead to experimentation in man. The major significance of the isolator system for rearing laboratory animals lies in the enhancement of detectable change in aging processes.

Proposed Course: The isolator contract is to be continued for a minimum of two years. Continuation will be dependent on commercial feasibility and colony survival rates.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: Unassigned

Contract Title: Summer Course in the Pathobiology of Aging

Contractor: Cornell University, Ithaca, New York

Money Allocated: \$39,806.00 (FY 1974)

Objectives: This contract is directed toward the development of a course on the pathobiology of aging that will provide the novice and the established investigator in aging with a basic understanding of the significance of microbial disease, pathology and degenerative change to the study of biological aging. The course is designed to enable experimental biologists in aging research to become familiar with the diseases and pathology of laboratory animals as well as systems and cellular pathology that may influence, simulate or replicate processes of aging. The course will bring together laboratory animal scientists and experimental pathologists with established expertise in microbial disease, systems and cellular pathology with a selected group of recent and established investigators currently engaged in aging research in mammalian model systems. Through lectures, seminars and interpersonal exchange with the faculty the course will provide investigators in aging with a basis for evaluating the significance of microbial disease, pathology and degenerative change on chronic studies in an atmosphere of open and free exchange between the experimental pathologist knowledgeable of abnormal biology and the experimental biologist concerned with the normal biological processes of aging. The format of the course is designed to enhance communication and consultation between the pathologist and biologist in aging by providing structured formal lectures to establish the basic concepts of disease and pathology significant to studies in aging followed by informal individual and group discussions that are aimed at developing a continuing dialogue between pathologists and biologists that will extend beyond the ten-day time frame for the course.

Significance for Aging Research: The study of aging is heavily dependent on observational studies of change in biological systems such as cells, tissues, organs and organ systems in animals. The material presented and the review and discussion of disease, pathology and degenerative change will provide young and established investigators in aging with an understanding of abnormal biology and disease and the implications these may have to the conduct of aging research, or for the isolation of cogent aging processes at the cellular, tissue, organ, organ system or organism level. Also, it should increase the understanding and interest of research pathologists in studies to define and differentiate biology of aging from abnormal biology.

NICHD Annual Report
July 1, 1973 through June 30, 1974

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 72-2755

CONTRACT TITLE: Quantitative Studies of Aging Human Diploid Fibroblasts
in Vitro

CONTRACTOR: University of Vermont
(Contract Officer: Dr. Marlene Absher)

MONEY ALLOCATED: \$51,000 (FY 1974)

OBJECTIVES: The Contractor is describing the division patterns and cellular lineages of human cells grown in culture utilizing time-lapse cinematographic, autoradiographic and computer analysis and model simulation techniques.

SIGNIFICANCE FOR AGING RESEARCH: The human diploid cell in culture is a widely-studied model for aging. Populations of these cells double actively under standard cell culture procedures for many months, but eventually age and die. Although extensive research is being conducted on populations of such cells, the studies are being pursued without definitive knowledge of the division characteristics of individual cells and their progeny. There is evidence that "old" populations contain many "young" behaving cells, just as "young" populations contain "old" behaving cells no longer capable of division. These and other data are being produced by Dr. Absher to the end of refining experimental design, cellular aging research concepts and hypotheses, and a capacity to relate cell culture studies of aging to the aging process in man.

NICHD Annual Report
July 1, 1973 through June 30, 1974

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 69-2091

CONTRACT TITLE: Production, Characterization and Distribution of Human
Diploid Cell Strains

CONTRACTOR: Stanford University, Stanford, California
(Contract Officer, Dr. Leonard Hayflick)

MONEY ALLOCATED: \$79,000 (FY 1974)

OBJECTIVES: This resource is designed to encourage the development of high quality research in the field of cellular aging by providing well-characterized cultured human cells to, as well as accelerated information exchange among the community of investigators studying the phenomenon of aging in the human diploid cell. Starter cultures may be obtained on a noncompetitive basis whereas more extensive needs for material are subject to merit review and competitive allocation of the resource. As individual laboratories acquire the capacity and need to produce their own cells, the demand on this contract facility decreases. Appropriate adjustments in funding level reflect such changes. Support for FY 1974 is reduced about \$25,000 from FY 1973 and an additional reduction of about \$30,000 is planned for FY 1975.

SIGNIFICANCE FOR AGING RESEARCH: This contract has been singularly effective in stimulating the development of a specialized field of research addressing cell aging phenomena in diploid human cells in culture. The contract has passed through the stage of being highly-speculative and of introducing new investigators to the field, to one where the major capacities of the resource are being consumed by investigators who are conducting long-term research on this cell strain and who depend upon this contract as a source of characterized and standardized cell materials.

Policy and substance of this contract have been subject to the review and deliberations of an ad hoc advisory panel which meets one or two times a year in addition to the formal contract review session.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: HD-2-2792

Contract Title: Prevention of Bone Loss in the Menopause

Contractor: Creighton University, Omaha, Nebraska

Money Allocated: \$47,000.00 (FY 1972) (Extended Without Funds)

Objectives: The purpose of this project is to study prospectively the effect on calcium metabolism and on bone mass of supplementing the diet with increased calcium or of administering sex hormones to women at or after the natural menopause. The study group consists of seventy-five nuns varying in age from 50 to 65 years. They will be assigned at random to three equal groups. One group is given calcium carbonate, the second group premarin with methyltestosterone, and the third group is a control. At the end of the second year of the project, comparisons of regression of bone mass as a function of age in the three groups will be accomplished to examine differences in rate of loss of bone. The study may provide information on the mechanisms by which these differences occur.

Significance for Aging Research: This study represents one of the first reasonably accurate assessments prospectively of the effect of combined estrogen-androgen replacement therapy on bone resorption and formation in postmenopausal women. Data correlating the effects of treatment with estrogen-androgen replacement or oral calcium on bone in the menopause is highly significant to assessing the effectiveness and need for replacement therapy in postmenopausal women. Thus, the studies supported under this contract have significance for understanding the changes that occur in bone mass with aging and will provide important information on calcium metabolic changes that result from therapeutic intervention in postmenopausal women.

Proposed Course: The contract to study prevention of bone loss in the menopause will continue for two years. Continuation is dependent on results from the first two years' study.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: HD-4-2756

Contract Title: Origin and Action of Estrogen in the Postmenopausal Woman

Contractor: The University of Texas Southwestern Medical School, Dallas,
Texas

Money Allocated: \$110,00.00 (FY 1974)

Objectives: The long-range goal of this project is to determine qualitatively and quantitatively estrogen production in postmenopausal women and the mode of interaction of estrogens and their target tissues. Earlier studies have shown, in oophorectomized postmenopausal women, that the principal estrogen produced is estrone and that for the most part, this arises from the extragonadal, extra-adrenal aromatization of androstenedione. In normal postmenopausal women most of the androstenedione is secreted by the adrenal glands but in women with ovarian stromal hyperplasia it can be secreted by the ovary as well. Also, changes in estrone have been shown to be associated with a variety of factors including obesity, age, diabetes, hypertension, liver function and some ovarian neoplasms. In view of the acquisition of this earlier data, the primary objectives of the contract are aimed at:

1. Establishing the relative contributions of ovaries and adrenals to plasma androstenedione and the extent of conversion of androstenedione to estrone and estrone sulfate prior to and following oophorectomy.
2. Determining the chemical nature of the hormone product following aromatization of androstenedione.
3. Delineating the effect of obesity, aging, hypertension, diabetes, liver function and some ovarian neoplasms on the conversion of androstenedione to estrone and estrone sulfate.
4. Determining the capacity of a variety of non-endocrine tissues to achieve the conversion of androstenedione to estrone by in vitro incubation studies.
5. Evaluate the relative efficacy of substances potentially active as aromatase inhibitors in an in vitro system utilizing a placental aromatizing enzyme system to inhibit excessive conversion of androstenedione to estrone, that occurs in some postmenopausal women.
6. Examining the interaction of estrone and estradiol in target tissues such as human endometrium and immature rat uterus.

With the attainment of these contract objectives, the origin and action of estrogen in postmenopausal women can be clearly defined as well as quality

and quantity of estrogen production.

Significance for Aging Research: The results of this study should contribute to understanding of the physiology and pathophysiology of estrogen production after the menopause as well as its medical management. Neither the quantitative nor qualitative characteristics of estrogen production in the menopause have been fully elucidated. It is clearly evident that the data on levels of endogenous estrogen production, the chemical nature of estrogens produced and their biological activity as well as factors which may contribute to alterations in endogenous estrogen production are needed. The acquisition of data under this contract is critical to understanding the degree of decline in estrogen production and the alternative methods by which estrogen production occurs in postmenopausal women. Also, a rational approach to replacement therapy in postmenopausal women cannot be developed until quality and quantity of endogenous estrogens produced during the menopause are clearly delineated.

Proposed Course: The contract is to continue for a minimum of one year.

NICHD Annual Report
July 1, 1973 through June 30, 1974

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: HD-3-2762

Contract Title: A Retrospective Study of Postmenopausal Women with and Without Estrogen Replacement Therapy

Contractor: University of California at Irvine, Irvine, California

Money Allocated: \$97,508.00 (FY 1973)

Objectives: The aim of the proposed epidemiologic study is to determine whether and to what extent estrogen usage tends to increase the risk of stroke in a population of postmenopausal women living in a retirement community. The contract provides for the support of a retrospective study of the incidence of cerebrovascular disease, and other factors which predispose to cerebrovascular disease in a specific and uniquely discrete population of 7,000 postmenopausal women. Cases of cerebrovascular disease occurring in postmenopausal women will be identified and described (morbidity and mortality). The population of identified stroke cases will be compared with an appropriate control group to determine if the case group is significantly different from that of the controls. Also, to determine the role of likely risk factors, proximity, dose and duration of drug usage in affecting severity, or type of stroke.

Significance to Aging Research: Until recently, the long-term effects of estrogens in postmenopausal women have been a matter of conjecture or largely ignored. The proposed study will provide current data on comparative risk of cerebrovascular disease in postmenopausal women taking estrogens and postmenopausal women who are not using them, but are otherwise at comparable risk from other causes.

The significance of this project lies in the fact that a substantial percentage of the women in the population are taking estrogen-like medications and the initial goal of the study can be completed within a two and one-half year period. At present the value of estrogen-like medication as therapy for postmenopausal symptoms, for the prevention of vascular disease, and for the arrest of osteoporosis remains unproven. There is serious concern that such medication in commonly used dosage is a significant health hazard with specific reference to the three most common causes of death--heart disease, cancer and stroke. There are no data available on the questions posed by this proposal. The population selected is uniquely constructed to permit this type of investigation. Information of this type is extremely important, since it may serve to guide the medical care of the many millions of women over the age of 50 in the United States.

Proposed Course: The study is planned for a minimum of one year. Continuation is dependent on results from the first two years' study.

NICHD Annual Report
July 1, 1972 through June 30, 1973

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 72-3-008

CONTRACT TITLE: Analysis of Aging-Associated Involution of Cellular Immunity
in Mice

CONTRACTOR: University of Florida, College of Medicine, Gainesville, Florida
(Contract Officer: Dr. Perry O. Teague, Department of Pathology)

MONEY ALLOCATED: \$6,500 (FY 1974)

OBJECTIVES: The Contractor will exert his best efforts to:

- 1) Determine whether or not thymic epithelial cells and/or uncommitted bone marrow lymphocyte stem cells decline in ability to function in normal cellular immune responses with aging;
- 2) Determine if an active thymus is required for retention of "normal thymus dependent cellular immune functions" during aging of thymectomized and of thymectomized-and-sublethally-irradiated mice;
- 3) Determine whether thymic epithelial cells or bone marrow stem cells from young and old syngeneic donors will reconstitute thymectomized and lethally irradiated mice of three strains: A/J, C57BL/6J and DBA/1J and
- 4) Determine if spleen cells from each mouse strain respond to stimulation in vitro to PHA, SEB and allogenic mitomycin-C-treated spleen target cells.

FINDINGS: Preliminary Results with Respect to Objectives

- 1) Irradiated and reconstituted thymectomized A/J mice: thymectomized mice had no residual thymus tissue. The spleen cells from old mice were more responsive to PHA than experimentally-reconstituted groups; responses to SEB and allogenic target cells were similar. Lymphoid function of A/J mice as measured by PHA responsiveness was significantly reduced in the thymectomized or thymectomized-and-irradiated groups. In contrast, T-cell function in all groups was the same when analyzed by responsiveness to SEB or allogenic target cells. No differences in peripheral blood lymphocyte counts were seen among groups. Results of studies with C57BL/6J mice were somewhat different from A/J mice two months following adult thymectomy. A significant decline in responsiveness to PHA was seen in the thymectomized, thymectomized-and-irradiated, and sham-thymectomized groups. Responsiveness as measured with SEB and allogenic target cells was inconclusive. Data from the DBA/1J mice two months after thymectomy were inconclusive.

- 2) An effort was made to determine the number of T and B cells in normal and adult thymectomized C57BL/6J mice. Normal animals were found to have 30% theta positive T-cells while the thymectomized animals were found to have 7% in the same population. In contrast, B-cells increased from 27% in normal animals to 36% in thymectomized animals.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND AGING PROGRAM: There is evidence that immunologic competence declines with age. Since immunologic diseases -- neoplastic, autoimmune and infectious -- show increased incidence with age, it is important to understand the genesis of this age-related decrease in immune competence. The inability of the organism to control such diseases is likely associated with a functional change in immunologically-reactive cells as well as with a decrease in ability to mount a rapid and intense immune response as would be required for infectious diseases. Studies should be encouraged to attempt to determine the mechanisms by which immunocompetence develops, the variables that modify the rates of its development, the significance of loss of competence and possible methods of therapeutic intervention.

PROPOSED COURSE: This contract should be maintained active through June 30, 1974.

NICHD Annual Report
July 1, 1973 through June 30, 1974

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Y01 HD 31003

CONTRACT TITLE: Longitudinal Interstudy Program

CONTRACTOR: Veterans Administration
Boston, Massachusetts
(Contract Officer: Dr. Benjamin Bell)

MONEY ALLOCATED: \$8,000

OBJECTIVES: This contract supports a series of meetings involving investigators of longitudinal studies of aging. The purpose of the meetings is to discuss topics of common concern to investigators of longitudinal studies, such as procedures and problems in collecting and utilizing longitudinal data. Each of the meetings is devoted to a particular topic and involves a small number of working investigators who share a common interest and responsibility.

MAJOR FINDINGS: Interstudy investigator teams have been established which deal with either methodological or substantive research questions. The investigator teams are in the following areas: cardiovascular aging, definition of health and disease, special senses research, psychological and sociological variables and their relation to biological variables, guides and models for large scale interstudy collaboration.

SIGNIFICANCE FOR AGING RESEARCH: Comparisons of procedures and data would do much to clarify the important differences and similarities among the various study populations. The conferences serve as a coordinating mechanism for longitudinal studies on aging.

PROPOSED COURSE: This contract should be maintained on a yearly basis.

NICHD Annual Report
July 1, 1973 through June 30, 1974

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 72-2767

CONTRACT TITLE: Review, Analysis, and Evaluation of Models for Forecasting
Future U.S. Population

CONTRACTOR: Duke University
Durham, North Carolina
(Contract Officer: Dr. George Myers)

MONEY ALLOCATED: \$92,000 (FY 73)

OBJECTIVES: This contract supports a review that will identify existing population models as well as classify and evaluate them. The aim is to develop predictive models that will be responsive to current needs and provide more sophisticated approaches to population planning for the elderly.

MAJOR FINDINGS: The effects upon population of eliminating major causes of death have been examined. Four causes of death were treated: major cardiovascular-renal diseases, influenza and pneumonia, malignant neoplasms and motor vehicle accidents. The projected population age 65 and over for the year 2000 resulting from the elimination of major cardiovascular-renal disease was nearly 75% greater than what would have resulted if this disease had not been eliminated. The corresponding difference for the projection eliminating malignant neoplasms was only 14%.

SIGNIFICANCE FOR AGING RESEARCH: Basic to the process of planning for the social, economic and health of the older population is an adequate understanding of its future size and composition.

PROPOSED COURSE: The ADA Branch will continue to support this research.

NICHD Annual Report
July 1, 1973 through June 30, 1974

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: NIH 74-C-323

CONTRACT TITLE: Summer Course on Psychological and Social Aspects of Aging

CONTRACTOR: University of Washington
(Contract Officer: Dr. Carl Eisdorfer)

MONEY ALLOCATED: \$42,759

OBJECTIVES: The course will bring together established investigators in the psychological and social sciences, and a cadre of young investigators to discuss and exchange information relevant to aging research. The purpose is to stimulate more research on the psychological and social aspects of aging.

PROPOSED COURSE: This contract should be awarded periodically to various institutions.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Mental Retardation Branch

Perhaps no other single problem within the broad range of human development is more complex and challenging than that of mental retardation. No other problem more clearly reflects the broad mission of the Institute, for mental retardation is a life-span problem requiring for its solution study of the full range of developmental variables.

The inadequately developed intelligence which characterizes the retarded individual is present early in life and obtains through maturity. Throughout its course, mental retardation impacts heavily on the individual, his family, community and the economic life and resources of our nation. The complex phenomena subsumed under the term mental retardation stem from many different causes and are expressed most commonly as the product of multiple biological, behavioral and social variables in interaction.

The task of bringing this wide and diverse range of phenomena together as a coherent body of related elements is similarly complex. Central to this effort is the need to effect synthesis of the many separate but related variables underlying the retarded condition which can give direction toward solution of the problem. Within the Institute, this task is assigned to the Mental Retardation Branch.

The primary mission of the Mental Retardation Branch is to develop and support research aimed at the prevention of mental retardation and, when this is not possible, to effect its amelioration. With the Institute, the Mental Retardation Branch is now approaching the end of its first decade of mission directed effort. This annual report presents an opportune time for review and appraisal of the Branch's work.

The history of the Branch is one of steady growth. In its first year, the program held 59 grants and contracts for a total outlay of \$2,748,000. In January, 1974 the number of grants and contracts had increased to 137 at a total level of support of \$18,226,000. Because the Branch holds a large number of program projects, the number of grants and contracts reported for 1974 gives a misleading indication of the number of discrete research projects supported. Each program project consists of several different but related projects. Taking this feature into account then, tabulation of all discrete research projects supported shows the number to be 247.

Numbers of grants and funding levels are but two dimensions reflecting program growth and activity. Program growth is also reflected in an ever widening of research problem coverage. Early in this decade the program had narrow coverage with grants concerned with inborn errors of metabolism dominant in program holdings. Today, program supported grants and contracts cover virtually every area of mental retardation research concern.

Legislation was passed (P.L. 88-164) in 1963 authorizing \$26 million in construction grant awards for centers for research on mental retardation and related aspects of human development. These Mental Retardation Research Centers (MRRC's) were heralded as primary agents for the nation's major thrust in the attack on mental retardation. Responsibility for this construction grant program was assigned jointly to the Division of Research Resources and Facilities, NIH and the Institute. Continuing responsibility for center program development and implementation was given to the Institute and its MR Branch.

The center construction effort has occupied the full span of this decade. The first constructed center was completed and occupied in 1968, four years after initiation of the construction phase of the program, and the last center became operational last year, 1973. In anticipation of the lag between initiation and review of center construction grant applications and completed construction of centers, staff initiated program efforts to bring the centers into productive operation prior to completion of construction. This was accomplished through programing efforts with the "paper staffs" listed on the construction grant protocols and by organizational work with the designated center directors.

These early discussions resulted in the recommendation, approved by Council in June 1968, for establishment of the center grant. Center grants and the guidelines governing their award were conceived of as a means for strengthening center administration by adding to its functional identity and capabilities.

Establishment of the center grant mechanism carried with it the recommendation calling for establishment of the Mental Retardation Research and Training Committee (HDMR). Study of the problems of center administration indicated that a stable, continuing scientific review body committed to the center's successful accomplishment of mission was necessary.

The review charge made to the HDMR Committee was scientific review of all center grants and all program projects, training grants, and fellowships assigned to the MR Branch. In addition, the committee was charged with review and report to Council of the relevance of each center's research activities to their primary mission—"research on mental retardation and related aspects of human development."

The HDMR Committee, from its inception, has met its charge successfully and in accordance with the highest NIH standards for scientific excellence. Its growing experience with each center has provided in-depth awareness of each center's needs, capabilities, and limitations against which any one of its immediate review responsibilities can be discharged.

The program project grant has become increasingly a key element in building the MR Branch. These grants are particularly suited to the purpose of the MRRC's, for they provide a mechanism whereby multidisciplinary and interdisciplinary research can readily be forwarded. In particular, the program project offers the opportunity to effect a synthesis of related elements around a central problem or theme—an opportunity sorely needed in mental

retardation research because of the diverse and interacting variables that determine the developmental life course of retarded individuals. As a consequence of the advantages offered by the program project, program staff has turned increasingly to this grant mechanism for program development.

MENTAL RETARDATION RESEARCH CENTERS

The MRRC program represents a potentially unique resource for multidisciplinary and collaborative research between biomedical and behavioral scientists to better understand the complex causes, pathogenesis and modes of prevention, treatment, and amelioration of mental retardation. More than 600 investigators representing a broad range of basic, clinical, and applied sciences and nearly twice as many supportive personnel are engaged in this effort. This concentration of research talent in mental retardation and related aspects of human development is the nation's response to this major social problem.

An important adjunct to research is the testing of new ideas in practice, the clinical application of preventive and treatment modalities and the training of manpower to staff emerging service programs. The University-Affiliated Facility program is particularly well suited to this purpose. Ten of the research centers also have UAF's, frequently in the same building and occasionally under the same administrative direction. The two programs are generally closely integrated on most campuses, with potentially enriching benefits to both. The integration of research with service and training activities has the additional value of helping to direct the centers toward mission-oriented goals.

Concern with the human condition is further enhanced by center affiliation with public or private residential facilities for the retarded, school and day-care programs and university or community-based diagnostic clinics. In some instances (Washington, UCLA, Kansas, Fernald, Wisconsin, Peabody, North Carolina), the tie with state institutions is especially close, with investigators holding faculty appointments, occupying laboratories in the institution or working directly with retarded subjects in ward settings. These administrative arrangements have been encouraged by the Institute to bridge the traditional gaps between universities, institutions, and communities, to facilitate the dissemination of new knowledge and its translation into practice, and to expedite program relevance. While this goal is not yet fully realized, coordinating mechanisms and partial communication systems already in force, indicate that real progress is being made.

The Institute's development of innovative policies for the fiscal support of the centers has been a major contributing force to their growth and viability. The most significant policy, designed to help the centers carry out their legal commitment to conduct research in mental retardation and related aspects of human development for a minimum of 20 years, is the center grant.

The center grant, which includes support for administrative costs, common equipment and supplies, and other supportive services of the research program effects certain economies through the sharing of resources (computer services, vivaria, high-priced scientific equipment, electronics, machine

shop, information services, support laboratories, etc.). Many of these resources, which undergird center activities, are not readily obtained through regular grant mechanisms and can be justified only when they serve a large number of investigators.

The most significant aspect of center support, however, is the provision for support of new program development personnel for a limited time, and other costs related to the initiation of new program areas. Such support has greatly enhanced center recruitment capabilities and faculty appointment and has resulted in the commitment of many outstanding scientists to mental retardation research. Perhaps more than any other single category of support under the core grant, this category is largely responsible for helping the centers become centers of excellence and effective competitors for grant funds.

The spectrum of research studies being conducted in the MRRC's embraces every known major dimension of the problem. Illustrative achievements are included in the section on research activities.

RESEARCH ACTIVITIES

The Mental Retardation Program sponsors a broad attack on the problem of mental retardation ranging from studies in fundamental molecular biology, biochemistry, neurophysiology, and genetics to epidemiology, pathology, obstetrics, and pediatrics and to psychology, sociology and special education. Of primary concern to the Institute are inquiries into the basic causes and means of preventing mental retardation through research into the biological and behavioral processes which contribute to or influence the development of these disorders. The Program's research attack is based on a program of research grant support, creation and support of special research facilities and resources, dissemination of scientific information through support of scientific conferences, and through contract support of research designed to accomplish specified research goals. Many of the research accomplishments which the NICHD has supported have been achieved in the 12 Mental Retardation Research Centers for which the NICHD has programmatic responsibility. These Centers, located throughout the country, are uniquely capable of carrying out research in the complex problems of mental retardation by calling upon the skills of investigators in many disciplines. In addition, the Centers provide training sites for future scientists.

Genetics and Inborn Errors of Metabolism

In institutions for the mentally retarded today, 5 percent of the patients have inborn errors of metabolism, 10 percent have Down's syndrome, and 25 percent have central nervous system defects, many of which are inherited. A survey of an unselected newborn population has demonstrated that 1 out of 200 babies has a major chromosomal abnormality. In addition, about 5 percent have minor chromosomal defects, the significance of which remains to be determined. Most of these genetically determined conditions are associated with mental retardation or defects which impair a child's ability to achieve his optimal development.

The problem of lowering the number of mongoloid births in this country is of special interest to the NICHD. Mongolism, or Down's Syndrome, is a form of mental retardation known to be genetic in origin. Scientists also know that the incidence of mongoloid births rises precipitously among offspring of women over forty. The NICHD hopes to promote increased understanding of the risks in pregnancies among women of this age group and has prepared a series of pamphlets on this topic for public and professional information.

Progress has been reported this year in extending the use of amniocentesis in diagnosing, before birth, inborn errors of metabolism and Down's Syndrome--genetic deficits many of which cause mental retardation. The technique is also an invaluable research tool for studying basic mechanisms causing the inborn errors.

Investigators from the Cincinnati Children's Hospital MRRC have developed an animal model for study of phenylketonuria. The model involves the combined feeding or injection of increased amounts of phenylalanine and an inhibitor of phenylalanine hydroxylase. Using this model, learning deficits have been shown to occur in offspring of female rats fed the PKU diet between days 10 and 20 of pregnancy, although the deficit exceeded and could not be distinguished from that resulting from maternal malnutrition produced by pair-feeding of control animals. Neither inhibitor nor excess phenylalanin alone fed to pregnant animals produced learning deficits in offspring.

Biochemical data derived from studies employing this animal model serve to confirm previous reports that the behavioral and biochemical aspects of experimentally induced phenylketonuria are similar to those of the human disease.

Investigators at the City of Hope Medical Center in California have now carried out field tests to evaluate two new low-cost, simple methods for detecting the metabolic error of galactosemic newborns. These tests can be combined easily with current screening programs for phenylketonuria to provide widespread early diagnosis of both diseases.

Institute-supported scientists have also made progress in studying chromosomal abnormalities. Working at the Mental Retardation Research Center at Children's Hospital in Boston, Massachusetts, an investigator is using sophisticated methods to study what happens within the cell nucleus when the chromosome doesn't divide at the normal time or when genetic materials does not perform its reproductive function normally. These aberrations are being investigated to determine the effects on production of necessary enzymes. Also, by making artificial hybrids of cells and by manipulating the cells' environment the investigators hope to determine which parts of the chromosome control production of specific enzymes. Conceivably, this work could contribute to the possibility of replacing or repairing defective chromosomes.

Teratology

Preliminary evidence obtained from experiments conducted at the Cincinnati Children's Hospital MRRC suggest that doses of acetylsalicylic acid too low to produce gross CNS malformations produce behavioral impairments. The

learning impairment was attributed to functional brain damage resulting from in-utero exposure to salicylates. The damage was not accompanied by anatomic abnormalities of the CNS although minor malformations of the skeleton and viscera were observed. This and other work in behavioral teratology points to need for expanded efforts in this area.

Early Intervention

Evidence supporting the effectiveness of early interventions for improving the development of retarded children has recently been provided by investigators at the Experimental Education Unit of the University of Washington MRRC. Working with Down's syndrome children in age groups from birth to 18 months, 19-36 months and from 3-5 years of age, these investigators have been able to demonstrate significant developmental gains in cognitive, speech and language, motor and self-help performance at all age levels. These gains hold up under sustained programming to school age using a methodology for preschool classroom training and parental instruction in intervention procedures. The most marked gains were achieved in those children for whom intervention began early in their development.

Malnutrition and Learning

The effects of malnutrition on the normal growth and intellectual development are still somewhat undefined, although thought to be widespread. A recent Institute-supported study done in cooperation with the government of Jamaica, West Indies, has shown that children who have experienced severe malnutrition during the first two years of life have lower intelligence levels at school age than their siblings and classmates. Because most brain development is completed before the age of nine months, children experiencing severe malnutrition before that age were compared with those affected later. No significant age-related differences could be observed. The finding that the malnourished children have lower intelligence is in accordance with the observation of other investigators. On the other hand, it is surprising that there is no observable association between intellectual level and age (up to 2 years) at which malnutrition occurred. The investigators point out that development of other nervous system tissue extends into later infancy. Accordingly, the child's intelligence remains vulnerable to malnutrition even after nine months of age. In light of this, the grantees suggest the need for studies of children who have experienced malnutrition at later ages.

Amelioration and Treatment

Growing disenchantment with the quality of residential care in the U.S. has led to administrative pronouncements to depopulate these facilities and to develop alternative patterns of care. Unfortunately, knowledge is not yet available as to the type of care most suitable for specific individuals and the program ingredients most likely to maximize development and adaptive behavior.

A large scale project at the Pacific State Hospital (a part of the MRRC at UCLA) is focusing on these issues. Efforts are underway to determine what criteria are applied in the selection of resources for placement, to

specify the nature of the environment and to evaluate outcome behaviors. Through research of this kind, it is hoped that movement of individuals from institutional to community-based facilities and the selective placement of children and adults in a variety of care, training, and rehabilitative settings can proceed on a sounder basis than heretofore.

Further, epidemiological studies have examined public institution and community residents. Data which have been collected on more than 20,000 residents with respect to diagnoses, adaptive behavior, physiological traits, social relationship and psychological characteristics probably represent the most comprehensive information available on institution populations. This data has been related to mortality, life expectancy tables, and training outcomes and has been applied effectively to institutional management and programming. The community study at Riverside highlighted the discrepancy between measured intelligence and adaptive behavior for ethnic minority groups. Many children of Mexican-American descent and bilingual in speech tested poorly on standard intelligence tests because of deficiencies in the English language rather than intellectual impairment. These findings stimulated policy changes in the educational system and the decertification as mentally retarded of thousands of children.

The activities of the Mental Retardation Research Centers encompass a wide range of basic, clinical and applied efforts including behavior modification technology. At the University of Kansas Mental Retardation Research Center, parents and scientific investigators collaborated to bring laboratory techniques for altering or eradicating undesired behavior into the home setting. Behavior modification is a system designed by scientists either to encourage desired behavior by rewards or to eliminate undesired activities by ignoring them or highlighting an alternative. Previously this technique had been used only in well-equipped laboratories on severely retarded children. In a recent center report, one parent noted that she was able to increase the frequency with which her son would wear his orthodontic device by giving him a reward if the brace was in his mouth at spot-check times. A second parent gave her daughter points and point trade-in items as an incentive to assist with household chores. The parent of a 4-year-old boy managed to significantly reduce the child's whining, crying, and complaining behavior by refusing to respond to it. And, in the fourth case, a mother decreased the amount of time it took her 5-year-old daughter to dress herself in the morning by making television privileges contingent upon promptness. These behavior problems were not severe enough to impel the parents to seek outside advice; they were, however, typical of those found in many homes that are common sources of friction.

As part of a large study of the relationship between maternal behavior and children's intellectual development, an Institute-supported scientist at UCLA collected data on child-rearing practices fostering optimal development. The investigator observed the effects of the mother's physical and vocal contacts and how much she ignored or played with the child. He found that the amount of contact between mother and child controlled not only the babies' responses to their mothers but also how much they stimulated themselves. The more the baby responded to its mother the less responsive it was to strangers. Also the

more suppressive or critical the mother was observed to be, the less socially responsive the baby was. Studies such as these provide data on child rearing practices which may affect children's development and make some children seem mentally retarded. The studies may also provide a basis for recommending certain practices which are shown to enhance development.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NIH-71-2447

Contract Title : Diagnostic and Intervention Studies of High-Risk Infants

Contractor : Regents of the University of California, U.C.L.A.

Money Allocated: \$641,903, FY 73

Objective : The purpose of this contract is: (a) to develop new diagnostic techniques and relate these to established ones, so as to best delineate those aspects of psychophysiological functioning in high-risk infants that indicate continuing risk for development, and (b) to ameliorate or modify this risk status through a program of specified interventions implemented from ten to twenty-four months of age.

Methodology : This prospective study is designed to specify those behaviors of high-risk neonates and infants which relate to performance on tests at two years of age. The study design is longitudinal, and correctional, covering a two-year period in each child's development.

The study sample of 200 subjects will be drawn from Los Angeles area hospitals and the UCLA Newborn Nursery and will consist of all premature infants of 37 weeks gestational age or less. The control subjects, full-term infants (39-41 weeks gestation) will also be drawn from the UCLA Newborn Nursery.

In the newborn period the infants will be rated on several behavioral and physiological diagnostic tests as well as on obstetrical and neonatal events. In addition, the infants's performance on other diagnostic tests will be assessed during the first four months. All infants and their parents will be provided with intensive medical, nursing, and social work support and anticipatory guidance in the nursery, well-baby clinic, and in the home.

When the infant is four months of age, the scores on all measures will be weighted and summed to derive a 4-Month Cumulative Risk Score (CRS). The CRS will then be used to classify the nine-month old subjects as high or low risk.

Additional diagnostic tests of behavioral and physiological functions will be administered during the four to nine months period. Medical, nursing, and social work support will continue for all babies both in the well-baby clinic and in the home. Scores on all of the diagnostic tests will be weighted and summed to yield a 9-month CRS. The 9-month CRS will be used to validate the risk and non-risk status of a 4-month CRS. At ten months of age, the high-risk infants, as determined by their CRS's, will be randomly assigned to an educational intervention or to a non-intervention group. Intervention will continue with the infants in the intervention group until the infants are two years of age. The diagnostic measures and the specialized intervention program will be evaluated by testing each child at two years of age on a variety of measures.

Results : The project is in its third year of operation. Pilot work is near completion and experimental subjects are being accepted into the formal research program.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NICHD-72-2794

Contract Title : Definition of a Behavioral Phenotype in the Cornelia deLange Syndrome

Contractor : The Regents of the University of California, San Diego

Money Allocated: \$123,895 FY 72

Objectives : A description of the general behavioral repertoire of patients with Cornelia deLange syndrome will be developed. This study will describe, codify, and quantify preliminary impressions that there is a characteristic behavioral pattern in this syndrome. Uniformity and consistency of observed movement phenomena will be examined and the distinctiveness of the behavioral phenomena to Cornelia deLange syndrome will be assessed. Determination will also be made of the degree to which the developed quantitative indices of behavior are useful in discriminating between different clinical conditions.

Methodology : Nonverbal behavioral phenomena such as general body activity, body posture, gait, posturing of extremities, facial muscular movements, and social interactions will be recorded on videotape in children with Cornelia deLange syndrome. As a result of experience obtained during the initial videotaping of five patients, the videotape recording protocol has been revised and will include the following general sequence: non-social sequence where the subject is led into and left in the studio containing a chair, small cot, and toys. After about four minutes, a sequence of 100 dB speech noise is activated for a few seconds on five different occasions at one minute intervals; social sequence with an adult stranger; social sequence with a familiar person; and twirling chair sequence. In each session, parents or those caring for the child will be permitted to view the entire recording. An extensive interview with the parents focusing on behavioral aspects and developmental details, will be taped at the end of each of recording session.

Results : Three additional patients with Cornelia deLange syndrome were observed and videotaped in San Francisco during the

past year using the revised protocol. Four patients from the Chicago area were evaluated and videotaped in Miami. These observations indicate that useful information can be obtained in this fashion. Commonalities of behavior are emerging which are interesting in that they affect not only the patients' response to his mother, but conversely her response to the child. These interactions may affect the entire family. Similar behavioral analysis could be applied to other retardation syndromes. The investigators are excited about the possibility that the results of this study could provide guidelines that would permit doctors to help parents learn how to live with an infant or child with this problem. In addition, following the observation that patients with Cornelia deLange syndrome became more tractable after bouncing and twirling, a more concerted evaluation of certain physical parameters such as tomograms and histopathological evaluation of the ear will be carried out.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

- Contract Number: 1-Y01-HD-30001-00 Interagency Reimbursable Agreement with the National Aeronautics and Space Administration
- Contract Title : An Automated System for Chromosome Analysis
- Contractor : Jet Propulsion Laboratory, California Institute of Technology
- Money Allocated: \$342,710 FY 72
- Objectives : The purpose of this contract is to develop an automated system for analysis of conventionally stained as well as "banded" chromosomes. The following goals are to be accomplished in this contract: development of a semi-automated system for the preparation of metaphase chromosome spreads on microscope slides; development of an automated light microscope system for fast, reliable, and efficient location and digitization of metaphase spreads; development of computer programs for karyotype analysis and classification of the digitized spreads; development of an interactive data management and analysis system for a cytogenetic data file; and systems testing using slides obtained from different groups of subjects.
- Methodology : The contractor will perfect an automated light microscope system that they have developed. The system examines microscope slides of human metaphase spreads which includes an object locator technique employing a rotating mirror, optical scanner, automatic focus procedure, and necessary programs for analysis of the image of selected metaphase spreads stained conventionally and using various "banding" techniques. The signal received by the optical scanner is sent through an analog to digital converter to a computer where, under interactive control of an operator, digital images of selected metaphases are written on tape for computer analysis. The final computer-generated karyogram is presented in the form of a high quality, hard copy print. Corresponding numerical measurements are available from the line printer.
- Results : The contractor has completed the design of the system and taken delivery on the bulk of the equipment needed to complete the contract. Progress toward the achievement

of all the goals as proposed in the contract is generally proceeding on schedule. The hardware selection has been sensible and the software design, apart from banding analysis which needs some modification, is reasonable. The engineering problems are surmountable and typical to any project of this type. The contractor is considered to be making progress within the production schedule.

Systems evaluation will include analysis of banded chromosomes obtained from 1,000 newborn infants; compare analysis of subset of cells done by at least two cytogeneticists and their (manual) analysis with the computer system; compare the karyotypes of known specimens containing both normal and abnormal karyotypes, with the computer classification.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NICHD-71-2081

Contract Title : Intellectual and Behavioral Consequences of Severe Malnutrition in Infancy: A Collaborative Study

Contractor : Albert Einstein College of Medicine

Money Allocated: None

Objectives : The aims of this study are to assess the relationship between severe malnutrition during the first two years of life in children and their subsequent intellectual development and behavior. Control groups of siblings and classmates are being compared on variables related to somatic, neurological, physiological, intellectual, educational, and family characteristics.

Methodology : This is a retrospective study of children aged 6-10 years who were hospitalized for severe malnutrition before two years of age. Detailed clinical records were kept of these infants regarding clinical severity of illness, duration of stay and somatic measures. In the follow-up study information on the index children and controls was obtained on a wide range of developmental and behavioral indices, school behavior and achievement, child rearing and social environmental stimulation.

Results : This project is now complete, although a final report of the data is not yet on file. A number of papers have been published indicating that the index children; namely, those subjected to severe malnutrition in infancy are shorter, weigh less, have smaller head circumference, lower IQ's, lower school achievement, and less satisfactory behavior in school. Both malnutrition and the lack of social stimulation appear to play relatively equal roles in the negative outcomes noted. Variations in the age of hospitalization from infancy to two years, did not appear significant in affecting developmental outcomes.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: N01 92180

Contract Title : Prevention of Prematurity and Developmental Disorders
Through Nutritional Supplementation and Laboratory Studies
on Prenatal Malnutrition

Contractor : Columbia University School of Public Health

Money Allocated: \$568,514, FY 73

Objective : The purpose of this contract is to carry out a controlled study on the effects of nutritional supplements to pregnant women in the socioeconomically deprived population of Harlem in New York City. Evaluation will be made of the impact of these supplements during various stages of pregnancy on birth weight, infant development, infant mortality and related disorders. Subjects will be drawn from a clinic population considered at high risk of giving birth to premature infants. This project is presumed to have great social and practical significance for improving physical and intellectual development in racial minority and poverty groups and for the prevention of mental retardation and related handicapping conditions.

The laboratory studies are designed to obtain a better understanding of the physiological parameters of growth and development and the potential role of infectious processes in low birth weight and retarded development. The correlation of data from these studies with pathological and clinical findings and data derived from the basic project will strengthen and extend the results and validity of the nutritional experiment.

Methodology: Experimental and control groups will be selected from a clinic population serving the Harlem area according to certain indices of high risk in producing low birth weight infants such as prepregnancy weight, previous history of prematurity, weight gain during pregnancy and age of mother and low protein intake. Other relevant factors including smoking habits, drug usage and trimester of entry into the project will be considered in the analysis. Infants will be assessed developmentally at birth, 26 and 40 weeks of age.

The laboratory studies include biochemical measurements of DNA, RNA and protein on placental and autopsy material and their relation to cell size and number. Morphometric measures will be made on by-products of pregnancy and infant tissues and studies of infection will be undertaken on maternal and infant serum and cultures.

Results :

The project is now entering its fifth year and has completed its full subject enrollment as of September, 1973. On the basis of interim evaluation and incomplete data, the number of subjects was reduced to approximately 900 and all of the babies from this cohort have now been delivered with a significant proportion having completed the final developmental tests at age 44 months. Data on outcomes for the total study are not yet available but preliminary analysis suggests that nutrition supplementation has been effective in increasing birth weight. The contractor is proposing a research grant application for the follow up of children at age 4 years to assess more reliably the relationship between intellectual and behavioral development and birth weight of children in the study.

The supplementary laboratory work initiated in February, 1972 is being carried out successfully according to the original protocols presented.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NO 1-HD-1-2446

Contract Title: An Investigation of Certain Relationships Between Hearing Impairment and Language Disability

Contractor : University of Washington, Seattle, Washington

Money Allocated: \$160,841, FY 73

Objective : The objectives of this project are best described in two phases. The three specific objectives of Phase I of this contract are: (1) to determine the feasibility of conducting an extensive investigation of the relationships between hearing impairment and the speech and language status of children from birth to three years of age; (2) to formulate a test battery for assessing the speech, hearing and language abilities of children under the age of 36 months; and (3) to develop specific testable hypotheses regarding the relationships between hearing impairment and language function. The results of Phase I of this contract will serve as a base for Phase II, a subsequent project on the investigation of the relationships between hearing impairment and language in young children. Phase II will provide a base for the investigation of language disability of children with other problems, such as cultural-familial mental retardation, physically based mental retardation, and cerebral palsy.

Methodology : The research will be conducted in four major areas: (1) auditory perception, (2) receptive and expressive language, (3) speech production, and (4) communicative interaction. In the area of auditory perception four facets will be examined: (1) behavioral responses to different auditory stimuli will be examined utilizing a variety of play techniques and conjugately programmed stimuli with a specially constructed manipulanda; (2) speech sound discrimination of 2-3 year olds will be tested using a key-foil discrimination task; (3) auditory sensitivity, examining behavioral responses using a population of 6-24 month old infants; and (4) auditory sensitivity, temporal integration and discrimination between paired auditory stimuli will be examined in infants using modifications of averaged electroencephalic audiometry.

Receptive and expressive language will be examined in terms of phonological, morphological and syntactic development

using transcriptions of language samples recorded on video tape. Speech production will be studied by (1) describing the vocalizations of infants in terms of feature networks, (2) describing the prosodic skill development in both normal and hearing impaired children under the age of two, and (3) measuring such physiological aspects of speech control as lip movement, jaw movement and lung volume. Communicative interaction will be examined using video tapes obtained in the child's home and evaluated for communicative and interactive behaviors. The tapes will be studied for number of vocalization-verbalization behaviors, communicative events, antecedent events, consequential events and behavioral events.

Results

: This project is in its third year of operation. During this period substantial progress has been made toward accomplishing each of these goals. The contract team has not only developed a set of testable hypotheses concerning the relationship between hearing loss and language development, but is attempting to develop a common theoretical framework in which to study receptive and productive processes.

The contract team's work on the development of techniques for evaluating hearing and language have also shown adequate and positively accelerating progress. They have adapted behavioral techniques for evaluating the hearing of children down to about 12 months and now are adapting instrumental conditioning techniques to study auditory sensitivity, sound discrimination, and sound recognition of the younger infants. The electroencephalic audiometry research has produced several interesting findings, but it now appears that this procedure will not be included in Phase II. The research on phonological transcription and analysis of the acoustic output of young children also shows progress. The group has realistically restricted their study of language primarily to the study of phonology with only a limited amount of work on semantics and syntax. A broader study including semantics and syntax at this time would lead to diffusion of effort. These three aspects of language can be combined in Phase II. The contract team has already published several papers in the methodology area.

Several methodological problems have been solved in the communication interaction area and data have been collected in the home setting with a video-tape sampling procedure developed by the contract staff.

Although the contract is still in the developmental phase, the work done is promising and unique insofar as it provides an intersection of several rather important areas of investigation. First, the group is making an attempt to integrate work on the development of auditory reception and vocal production within a common framework; namely, the framework of rhythm being an organizing principle for language development. Second, they are investigating these relationships with very young children. Third, they are introducing these sophisticated and promising techniques of study into the area of early language development of the hearing impaired and retarded populations.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NIH-72-2016

Contract Title : The Development of Materials for Application of Behavior Modification Skills by Parents of Mentally Retarded Children

Contractor : Behavior Education Projects, Inc.

Money Allocated: None

Objectives : The purpose of this contract is to test the efficacy of printed materials as used by parents, in shaping the behavior of their retarded children. Determination will also be made of the degree of supplementary training which may be necessary in the use of such materials and the relationships between child and parental characteristics and training effectiveness.

Methodology : This project involves several phases of operation. Initially, parents will identify problems and training needs posed by the behavior of their children and programmed texts will be developed responsive to these needs. After these materials have been pretested, controlled experiments will be undertaken varying the use of the texts and degrees and form of supplementary training. Parent and child characteristics will be included in the analysis of the data.

Results : This project initiated in October, 1971 has been completed and a final report published. A total of 13 program texts in the area of self-care skills, speech and language, and behavioral problems have been developed and pretested and distributed in response to numerous requests for this material. The experimental phase of the project has resulted in the finding that parents utilizing the manual materials alone have been just as effective, if not more so, than the parents receiving the manuals and supplementary home visits and professional training. Intermediate groups to whom limited supplementary assistance was provided were less effective than the manuals only group. Cost benefit analysis indicated a difference of \$38 to \$211 per child between the two extremes of the experimental groups, a finding of practical and social significance. Profiles of the characteristics of the parents suggest that social class was not a discriminating factor in the utilization of the materials, although the lowest social class was not represented in this study.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: N01-HD-3-2713

Contract Title : Coordination of Regional Cytogenetic Registries

Contractor : University of Oregon Medical School, Portland, Oregon

Money Allocated: \$39,539 FY 73

Objectives : This contract will attempt to coordinate the existing cytogenetic registries in Oregon and New York, and a new registry in Denver, Colorado. In addition, a uniform operating procedure for acquiring, storing, and retrieval of chromosomal data will be developed.

Methodology : The Oregon group will obtain and code chromosomal studies performed primarily in the Medical School Clinical Cytogenetics Laboratory which includes 800 new studies per year. In addition it will log in previously acquired cytogenetic information on approximately 10,000 patients.

The Colorado registry will be responsible primarily for coding cytogenetic data from population surveys, patients exposed to background radiation, clinic patients seen in Denver with an expected annual data on about 13,000 subjects. The New York registry, in addition to continuing acquisition of cytogenetic data from the state of New York, will also collect data for coding from established cytogenetic laboratories in Vermont, Massachusetts, and Connecticut. Over 2,000 cases annually are expected to be logged in from this group.

The contract will develop a manual of procedures, establish defined standards in selection of material for analysis and for quality control, evaluate compatibility of the computer programs developed for use by the collaborating laboratories, and apply on a limited basis the common format and manual of procedures.

Results : This contract was initiated in June, 1973. An acceptable unified common format and a manual of procedures have been developed which includes pictures of fluorescent and Giemsa-banded karyotypes as models for quality control. The investigators have identified a few cytogenetic abnormalities which could not be accommodated in the existing nomenclature developed at the Paris Conference in 1971.

The nomenclature needs modification of its conventional terminology to accommodate newly described chromosomal abnormalities.

Compatibility of the different computers will soon be demonstrated. A more intensive application of the common format and the manual of procedures will be carried out. Based on satisfactory progress to date, the investigators have been encouraged to submit a request for extension of the contract for two additional years.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: None at this time

Contract Title : Social Aspects of Mental Retardation in Poland

Contractor : Columbia University School of Public Health

Money Allocated: \$60,000 , FY 73

Objectives : The goals of this study are to explore the relationships between cultural familial factors and social class in mild mental retardation without detectable organic impairment. Efforts will be made to factor out components associated with social stratification such as material conditions of poverty, housing and physical environment; access to education, health and use of facilities; nutrition; and such cultural factors as the socialization process, attitudes to schooling, etc. The situation in Warsaw, Poland provides a unique, experimental opportunity to isolate these variables and the confounding effect of social class per se because of the socialist regime which has markedly reduced long-standing income gradients between skilled, unskilled and professional occupation groups. Such information could provide a basis for national social action and policy aimed at prevention, therapy, and amelioration.

Methodology : The first phase of the study involves a determination of the prevalence of mild mental retardation in a 1962 birth cohort. Data will be collected from a finding of school and agency records and census tract data. Study and control subjects will be drawn from this birth cohort. The second phase will be an intensive investigation of identified groups of retarded children and controls in order to delineate their mental, psychological, and social characteristics. Psychological test instruments and interview techniques will be applied. Analysis of the data from the first phase will guide the focus of the substantive second stage.

Results : The pilot phase of the study was initiated in October, 1973 under a PL-480 grant from the Social and Rehabilitation Services Administration. This contract is to provide support for the data analysis aspects of the study, inasmuch as computer resources are not available in Poland and thus cannot be supported through the counterpart currency program. Preliminary data from the initial phase of the study will be partially analyzed during the coming year.

NICHD ANNUAL REPORT
July 1, 1973 through June 30, 1974
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NIH-74-2848

Contract Title : Follow-Up Study on the Application of Behavioral Modification Skills by Parents of Mentally Retarded Children

Contractor : Behavior Education Projects, Inc.

Money Allocated: \$23,927, FY 73

Objectives : This is a follow-up study of contract NIH-72-2016. The objective is to determine the degree to which parents in the various experimental groups of the initial study continue to apply behavior modification principles, knowledge, and materials in shaping the behavior of their retarded children. Since the original study involved relatively short-term training (five months), the duration of the effects of training poses important issues. Answers to this question will help to evaluate the long-range effect of various approaches to parental training and thereby, suggest strategies for follow-up programs of this kind.

Methodology : Contacts will be made with all parents participating in the various experimental groups of the original project. Interviews will be conducted and test instruments applied to assess the parents interaction behavior with their retarded children in influencing the development of behavioral skills and in the determination of their continuing knowledge regarding behavior modification problems and technologies. Approximately 100 parents will be involved in this follow-up study and data both on parent and child characteristics will be included in the analysis.

Results : This project was initiated on May 1, 1974 and a final report is expected in one year.

NICHD ANNUAL REPORT
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Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

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